



# Acta Obstetricia et Gynecologica Scandinavica

159 1980 No 1

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# Acta Obstetricia et Gynecologica Scandinavica

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## STUDIES IN NORMAL PREGNANCY — SERUM LIPIDS LIPOPROTEINS AND URIC ACID (II)

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**Abstract** Three different series of healthy pregnant women without any history or symptoms of metabolic disorder have been studied for serum lipids (cholesterol, triglycerides and phospholipids) as well as for cholesterol and triglyceride content in lipoprotein fractions in the 1st, 2nd and 3rd trimesters. Two other series of non-pregnant healthy women previously studied for serum lipids are presented for comparison.

In the 1st trimester serum cholesterol decreased ( $p < 0.1$ ) as compared to non-pregnant values. In the 2nd and 3rd trimester all serum lipids increased successively with maximum values during 3rd trimester. All serum lipids increased successively with maximum values during 3rd trimester. The changes were most pronounced in serum triglycerides.

Among lipoproteins cholesterol content increased in all fractions during the 2nd trimester and was most pronounced in very low density lipoproteins (VLDL) (in the 2nd trimester by 78 per cent and in the 3rd trimester by an additional 94 per cent). The increase in triglycerides in the 1st and 2nd trimester was most pronounced in infranantant (LDL+HDL) while in the 3rd trimester the triglyceride content was more evenly distributed among infranantant and supernatant (VLDL) at density 1.006 g/ml. It is suggested that the increase in triglycerides is due to increased liver synthesis in the 1st and 2nd trimester concomitant with an enhanced removal i.e. by increased lipoprotein lipase activity (PLA). In the 3rd trimester however LPLA is possibly inhibited by subclinical cholestatic changes in the liver causing a maximum triglyceride accumulation preferentially located in VLDL.

In an earlier study dealing with serum lipid changes in diabetic pregnancy marked hyperlipidemia in the 3rd trimester was a characteristic feature (23). Further more elevated serum triglycerides in the mother appeared to be related to an increased birth weight of her infant.

A long parallel study was needed in normal pregnancy to allow a comparison with the serum lipid variables in diabetes. The aim of the present investigation was to study serum lipids and lipoprotein distribution in normal pregnancy.

## MATERIAL AND METHODS

**Clinical material** Three different series of healthy pregnant women consecutively selected from the Maternal Welfare Unit: one series of non-pregnant healthy women and one series of postpartum women consecutively selected from the department of Obstetrics were studied.

**Series I (1st trimester)** Twenty pregnant women (mean age 24.4, range 14–40 years) with normal uncomplicated pregnancy were studied at 8–12 weeks (mean 10.6 weeks) of gestation.

**Series II (2nd trimester)** Twenty pregnant women (mean age 26.6, range 20–41 years) with normal uncomplicated pregnancy were studied at 20–24 weeks (mean 21.7 weeks) of gestation.

**Series III (3rd trimester)** Seventeen pregnant women (mean

**Table 1** Serum lipids (cholesterol, triglycerides and phospholipids) and uric acid at different stages of normal pregnancy. 1st trimester = week 10 (range 8–12) of gestation. 2nd trimester = week 22 (range 20–24) and 3rd trimester = week 34 (range 32–36). Serum lipid data are given in mg/100 ml

Lipid		1st	2nd	1st vs 2nd	3rd	2nd vs 3rd
Cholesterol	Mean	173	223		247	N.S.
	SEM	6	9		10	
Triglycerides	Mean	64	101		164	
	SEM	5	6		12	
Phospholipids	Mean	181	237	*	284	
	SEM	6	9		11	
Uric acid	Mean	2.55	2.43	N.S.	3.38	
	SEM	0.19	0.15		0.19	

\*  $p < 0.01$   $p < 0.001$  vs 1st

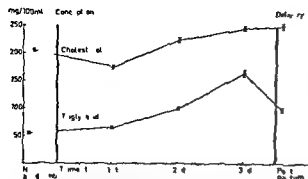


Fig 1 Serum lipids (cholesterol and triglycerides) at different stages of normal pregnancy in the non pregnant state and post partum 1st trimester = mean week 11 of gestation 2nd trimester = mean week 22 3rd trimester = mean week 34 and post partum = 2–5 days after delivery Number and age of patients see Material and Methods Data on serum lipids in mg/100 ml Mean  $\pm$  SEM

age 26.2 range 20–32 years) with normal uncomplicated pregnancy were studied at 32–36 weeks (mean 34.2 weeks) of gestation

Series IV (post partum) Ten normal post partum women (mean age 24.2 range 20–28 years) were studied 2–3 days after uncomplicated delivery following a normal pregnancy Series V (non pregnant) Eighteen non pregnant women (mean age 28.2 years range 19–34 years) with regular menstrual cycles and not using oral contraceptives were studied on the 1st or 2nd day of menstrual bleeding and served as controls

The women in the five groups did not differ in mean age had a normal body weight and the pregnant women a normal weight gain throughout pregnancy Data in series III IV and V have partially been presented previously (20–21) and are only included for the purpose of comparison Blood samples were drawn in the fasting state in the morning centrifuged at  $2500 \times g$  for ten minutes and the serum thereafter immediately recovered frozen and stored at  $-20^\circ\text{C}$  in glass tubes with teflon screw caps

**Serum lipids** Cholesterol was determined according to Cramer and Isaksson (3) triglycerides by the method of Carlson (4) and total phospholipids as described by Bartlett (1)

**Serum lipoproteins** Very low-density lipoproteins (VLDL) were isolated by ultracentrifugation

Centrifugation was carried out in a No. 50 Rotor of the Spinco Model L Ultracentrifuge at  $0^\circ\text{C}$  for 22 hours at  $105000 \times g$  The cholesterol (15) and triglyceride (4) content of supernatant and infranatant ( $D 1.006 \text{ g/ml}$ ) were determined The cholesterol content (15) of the  $\alpha$  lipoproteins ( $\alpha$  LP) was determined on whole serum after the elimination of VLDL and LDL by precipitation with heparin and manganese chloride (3) LDL cholesterol was estimated as the difference between cholesterol content of INF (infranatant) at density  $1.006 \text{ g/ml}$  and  $\alpha$  lipoprotein ( $\alpha$  LP) cholesterol

Uric acid was determined according to standard methods Statistical methods Conventional methods were used for the calculation of means standard deviations and standard

errors of means Student's *t* test was used to study differences between different groups and dependent *t* test was used when analyzing differences between means within groups When testing correlation coefficients between different variables due consideration was taken to the fact that some of them have a functional relationship (i.e. serum cholesterol and its components) Values of  $p < 0.05$  were considered statistically significant (2)

## RESULTS

### I Serum lipids in 1st trimester (Table I and Fig 1)

The present series of healthy pregnant women showed a decrease ( $p < 0.01$ ) in serum cholesterol in the 1st trimester as compared to non pregnant women

**II Serum lipids in 2nd trimester (Table I and Fig 1)** All serum lipids cholesterol ( $p < 0.001$ ) triglycerides ( $p < 0.001$ ) and phospholipids ( $p < 0.001$ ) increased in the 2nd trimester as compared to the 1st

**III Serum lipids in 3rd trimester (Table I and Fig 1)** Serum triglycerides ( $p < 0.001$ ) and phospholipids ( $p < 0.01$ ) increased to reach a maximum value in the 3rd trimester as compared to the 2nd

### IV Serum lipoproteins in 2nd trimester (Table I)

Fig 2 Serum lipoproteins expressed as distribution among VLDL (very low density)

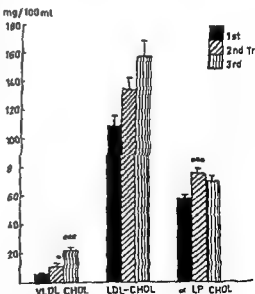


Fig 2 Cholesterol distribution among lipoproteins at different stages of normal pregnancy VLDL = very low density lipoproteins LDL = low-density lipoproteins  $\alpha$  LP =  $\alpha$  lipoproteins (for isolation see text) 1st trimester = mean week 11 of gestation 2nd trimester = mean week 22 3rd trimester = week 34 Number and age of patients see Material and Methods Cholesterol content in mg/100 ml Mean  $\pm$  SEM

$p = 0.05$   $** = 0.01$   $*** = 0.001$  level

Table II Lipoprotein triglyceride content at different stages of normal pregnancy Sup < 1 006 = very low density lipoproteins (VLDL) and inf > 1 006 = low-density lipoproteins (LDL) plus high density lipoproteins (HDL) 1st trimester = week 10 (range 8–12) of gestation 2nd trimester = week 22 (range 20–24) and 3rd trimester = week 34 (range 32–36) Triglyceride content is given as mg/100 ml Mean  $\pm$  SEM

Trimester	1st	2nd	1st vs 2nd	3rd	2nd vs 3rd
Triglyceride					
Sup < 1 006 (VLDL)	29 0 $\pm$ 3.2	41 5 $\pm$ 5.5	NS	89 2 $\pm$ 12.0	
Inf > 1 006 (LDL + HDL)	44 0 $\pm$ 3.7	65 7 $\pm$ 4.4		92 4 $\pm$ 5.1	*

0.05 0.01 0.001 level

ns) LDL (low density lipoproteins) and  $\alpha$  LP lipoproteins) increased in the 2nd trimester as compared to the 1st VLDL cholesterol increased by per cent ( $p < 0.01$ ) LDL cholesterol by 24 per cent ( $< 0.05$ ) and  $\alpha$  LP by 31 per cent ( $p < 0.001$ ) triglyceride content increased in the infranant (INF) 1 006 g/ml) i.e. in LDL + HDL ( $p < 0.001$ ) in the 3rd trimester as compared to the 1st

Serum lipoproteins in 3rd trimester (Table II and Fig 2) Lipoprotein cholesterol content increased in LDL by 94 per cent ( $p < 0.001$ ) in the 3rd trimester compared to the 2nd Triglyceride content increased in VLDL ( $p < 0.001$ ) and in LDL + HDL ( $p < 0.001$ ) in 3rd trimester as compared to the 2nd The total LDL-triglyceride and  $\alpha$  lipoprotein triglyceride as 2.1 (by weight) in the 3rd trimester

I Serum uric acid in the 1st 2nd and 3rd trimester (Table I) Serum uric acid increased in the 3rd trimester by 40 per cent ( $p < 0.001$ ) as compared to the 2nd trimester

II Correlations between serum lipids and lipoproteins (Table III) Serum cholesterol was correlated to LDL cholesterol content in the 2nd trimester ( $r = 0.57$   $p < 0.01$ ) and to LDL cholesterol content in the 1st ( $r = 0.93$   $p < 0.001$ ) 2nd ( $r = 0.95$   $p < 0.001$ ) and 3rd ( $r = 0.93$   $p < 0.001$ ) trimesters Changes in LP-cholesterol did not influence serum cholesterol to any major extent as judged from lack of correlation Changes in serum triglycerides mainly reflected changes in triglyceride content of VLDL as indicated by high correlation in the 1st ( $r = 0.81$ )  $p < 0.01$  2nd ( $r = 0.84$   $p < 0.001$ ) and 3rd trimester ( $r = 0.64$   $p < 0.01$ ) Serum triglyceride changes were related although to a lesser extent to triglyceride content in infranant 1 006 g/ml (LDL + HDL) in the 1st trimester ( $r = 0.64$   $p < 0.01$ ) and in the 2nd trimester ( $r = 0.54$   $p < 0.05$ ) There was no correlation between serum triglycerides and  $\alpha$  LP cholesterol There was also lack of correlation between serum uric acid and serum lipids

## DISCUSSION

The present data in agreement with similar studies (6 14 24) reveal a marked elevation in serum lipids in normal pregnancy This increase in serum lipids was greatest in the later stages of pregnancy Cholesterol increased were most pronounced in the 2nd trimester and triglycerides and phospholipids in the 3rd Serum triglycerides increased by 200 per cent (as compared to non pregnant values) in the 3rd trimester After delivery serum triglycerides declined within a few days to approach non pregnant levels (Fig 1) Two to five days after delivery however both serum cholesterol and triglycerides were still higher ( $p < 0.01$ ) than in the non pregnant state

In the 2nd trimester the increase in serum cholesterol was due to an increase in cholesterol content of all lipoprotein fractions i.e. VLDL LDL and  $\alpha$  LP This generalized cholesterol increase might be due to the effect on cholesterol metabolism either of increased synthesis or more likely of decreased catabolism Data on serum lecithin fatty acid composition (22) has been interpreted in the 2nd and 3rd trimester as indicating a subclinical cholestatic involvement of the liver The presence of increased bile acids in the liver in mild cholestasis would be expected to inhibit cholesterol catabolism (11) In the 1st trimester on the other hand a reduction in serum cholesterol might be the expression of an estrogen influence on liver metabolism since serum lecithin relative fatty acid composition in the 1st trimester would indicate an estrogen influence on liver lipid synthesis (22) Furthermore it has been well documented that the administration of estrogens in man decreases serum cholesterol (10 16 18)

Since serum triglyceride primarily reflect VLDL triglyceride content as judged by the high correlation in all trimesters it is suggested that increased triglyceride synthesis is the primary cause of elevated serum triglycerides during pregnancy Increased tri-

Table III Correlations (*r* values) between serum lipids and lipoprotein variables at different stages of normal pregnancy 1st trimester = week 10 (range 8–12) of gestation 2nd trimester = week 22 (range 20–24) and 3rd trimester = week 34 (range 32–36)

Trimester	1st	2nd	3rd
Serum CH vs VLDL CH	0.14	0.57	0.07
Serum CH vs LDL CH	0.93	0.95	0.93
Serum CH vs $\alpha$ LP CH	0.30	0.28	0.18
Serum TG vs VLDL TG	0.81	0.84	0.64
Serum TG vs (LDL + HDL) TG	0.64	0.54	0.43
Serum TG vs VLDL CH	0.14	0.88	0.90
Serum TG vs $\alpha$ LP CH	0.17	0.25	0.30

) 0.05 ) 0.01 ) 0.001 level

glyceride levels during pregnancy might be related to an increased level and turnover of free fatty acids (FFA) (7) to disturbances in carbohydrate metabolism associated with an elevated basal insulin level (hyperinsulinemia) (17) as well as to elevated growth hormone (STH) concentration (19–9).

During early pregnancy an increased synthesis would possibly be compensated by an increased triglyceride efflux by lipolysis and also by transfer of triglycerides from VLDL to LDL. In the 3rd trimester an elevated VLDL content of both cholesterol and triglycerides would possibly indicate that this efflux was partially inhibited. A decreased efflux might among other things suggest a decreased lipoprotein lipase activity (LPLA). In view of the suggestion of a subclinical cholestatic condition in the 3rd trimester (22) an inhibition of LPLA by bile acids would be a possible explanation.

A high triglyceride content in HDL (in the 3rd trimester 20 per cent of total serum triglycerides were found in HDL) would suggest the appearance of triglyceride rich associated or non associated lipoprotein families in HDL presumably LP C originating from the catabolism of VLDL. An alternate explanation for triglyceride rich HDL would be the appearance of abnormal LP A originating in the liver induced by an accentuated triglyceride (12–13) and protein synthesis (8). Studies in progress on the lipoprotein families of HDL will hopefully give an answer to this intriguing metabolic question.

#### ACKNOWLEDGEMENTS

Mrs Aina Lidell, Miss Inga Hvass and Mrs Margit Wettsten are acknowledged for technical assistance and Miss Annika Hofde for typing the manuscript. This project was supported by grants from the Swedish Medical Research Council (19X–2100).

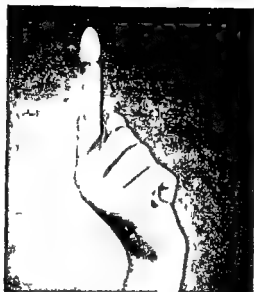
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Submitted for publication July 27 1978

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# INTERVILLOUS BLOOD FLOW IN NORMAL AND COMPLICATED LATE PREGNANCY MEASURED BY MEANS OF AN INTRAVENOUS $^{133}\text{Xe}$ METHOD

K Käär P Jouppila J Kuikka H Luotola J Toivanen and A Rekonen

From the Departments of Obstetrics and Gynecology University of Oulu and Central Hospital of Central Finland and Department of Clinical Chemistry University of Oulu Finland

**Abstract** Intervillous blood flow (IVBF) was measured intravenously with a new quantitative  $^{133}\text{Xe}$  method in 50 normal and 74 complicated late pregnancies between the 35th and 42nd weeks. The distribution of individual flow rates seemed to be fairly wide in both the normal and the pathological groups. The mean rate of IVBF in normal pregnancies was 140 ml/100 ml of intervillous space/min. The lowest mean flow values were observed in pregnancies complicated by diabetes mellitus (class B-E), cholestasis of pregnancy and severe pre-eclampsia, a highly significant difference ( $p < 0.001$ ) from the mean IVBF observed in normal pregnancies. The significance of the results in the different groups has been discussed in detail. This method may open up a new diagnostic area in the management of high risk pregnancies.

Intervillous blood flow (IVBF) is important for fetal nutrition, respiration and excretion (4) and in normal pregnancy it increases towards term (25). Changes in uterine hemodynamics and structural properties of the placenta may markedly alter the IVBF (3, 11, 25). The acute reduction of IVBF required to place the fetus at risk has been estimated to be fifty per cent (26). The reduced IVBF usually associated with intrauterine hypoxia may result in growth retardation, placental injury and occasionally even death of the fetus (26). Most of the radionuclide methods developed for the evaluation of IVBF have the disadvantages that they give only qualitative information (5, 16, 25). Recently a new quantitative method for measuring IVBF has been presented (20). This method is based on intravenous use of  $^{133}\text{Xe}$ . In order to evaluate this new method we studied IVBF in normal pregnancies and in patients with some of the most common complications of late pregnancy.

## MATERIAL AND METHODS

The series consisted of 124 pregnancies between the 35th and 42nd gestational weeks. The pregnancies were divided into six groups according to maternal diagnosis

(Table I). The White classification was used in diabetes mellitus. The case was considered one of dysmaturity if the weight of the newborn was under the 10th percentile and the pregnancy was otherwise normal (9). Fifty one of the pregnancies were studied in the University Central Hospital of Oulu and 93 in the Central Hospital of Central Finland in Jyväskylä. Fifty of the pregnancies were normal and they were used as a control group.

**$^{133}\text{Xe}$  method.** The placental site was localized in advance by means of ultrasonic B-scanning and only those with anterior wall placentas were included in the final series. During the examination the patient lay in a 15° C left lateral tilted position. The gamma camera head with a low-energy collimator (RADICAMERA) was placed over the placental area. A dose of 2.5 mCi of  $^{133}\text{Xe}$  saline (gamma radiation energy 80 keV, half life 5.3 d) was injected rapidly into the left antecubital vein through a two-way syringe and flushed immediately with 10 ml of physiological saline. To minimize the excretion of  $^{133}\text{Xe}$  through the lungs the patient was told to hold her breath for 15-20 s after the injection. For recording quantitative data from the placenta, a data storage (PDP 8, 12 k words) and read-out system with fast television techniques (NUKAB) was connected to the scintillation camera. The data in digital form were collected using 64 × 64 image matrices with 10 s channel width over 10 minutes. After the measurements were taken the data were transferred onto a magnetic disc. The total data density over the placenta ranged from 200 to 2 000 counts per  $\text{cm}^2$ . The peak count rate was 1 000-10 000 counts/10 s. The procedure used in Jyväskylä was the same with the exception that a function measurement system with two detectors was used. The method was reported in detail previously (20).

**Calculation.** The time activity curve of each area was fed off line into a central computer (NOVA 840, 64 k words) for processing. In Jyväskylä the detectors were connected on line to a NOVA 1220 minicomputer. A two-exponential function ( $A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t)$ ) was fitted to the measured wash-out curve using the least square method. The fast component describes the fractional removal ( $dQ(t)/dt = k_1 Q(t)$ ) of the tracer from the intervillous blood pool. The regional intervillous flow (in ml/100 ml/min) was calculated from the equation  $F_1 = 100 k_1$  and the myometrial flow from the slow component by using the conventional wash-out equation  $F_2 = p k_2$  where  $p$  is the partition coefficient between blood and myometrium (70 ml/100 g hematocrit 45 per cent). Myometrial blood flow was not recorded in this study.

Radiation dose to fetus was less than 1 mrad.



Table 1 The rates of intervillous blood flow birth weights of infants and placental weights in normal and complicated pregnancies

Diagnosis	N	Intervillous flow ml/100 ml/min		Infant birth weight (g)	Placental weight (g)
		mean±SD	range	mean±SD	mean±SD
Normal pregnancy	50	140±53	73-261	3 614±384	648±111
Essential hypertension	31	112±39	63-183	3 420±592	597±126
Severe pre-eclampsia	11	99±27	61-120	3 183±590	565±139
Diabetes mellitus (class A)	5	110±16	96-138	3 872±592	768±256
Diabetes mellitus (class B-E)	7	89±19	76-131	3 439±781	644±149
Cholestasis of pregnancy	8	94±14	66-114	3 350±425	600±92
Dysmaturity (<10th percentile)	12	109±34	64-164	2 482±537	440±101

= almost significant    = significant    highly significant

## RESULTS

The mean values, standard deviations and ranges of IVBF, infant birthweight and placental weight in normal cases and the different groups of complicated pregnancies are presented in Table 1. The mean IVBF was highest in normal pregnancies ( $140\pm53$  ml/100 ml/min) and lowest in diabetes mellitus (class II E) ( $89\pm19$  ml/100 ml/min). In essential hypertension the reduction of IVBF was almost significant ( $p<0.05$ ) and in all the other groups significant ( $p<0.01$ ) or highly significant ( $p<0.001$ ) if compared with the mean value of the control group. In severe pre-eclampsia the mean birthweight was almost significantly ( $p<0.05$ ) lower than in normal pregnancy and in dysmaturity the difference was of course highly significant ( $p<0.001$ ). In the other groups there were no statistically significant weight differences.

The percentage of dysmature babies (19) in the different groups were as follows: normal pregnancy 6, essential hypertension 23, severe pre-eclampsia 45, diabetes mellitus (class A) 0, diabetes mellitus (class II E) 14, and cholestasis of pregnancy 25.

Only in dysmaturity was the mean placental weight statistically lower than in normal pregnancies ( $p<0.001$ ). The foeto-placental weight ratios were normal in all groups ( $17\pm2$  per cent).

Seven of the infants had a 1 minute Apgar score of six or less, while all the others were in good condition.

We had one fetal loss in this series. The mother was a 30-year old primigravida with diabetes mellitus (class E) and superimposed severe pre-eclampsia. IVBF on the 35th gestational week, one week before the death, was only 75 ml/100 ml/min. The stillborn baby was very dysmature, weighing 1 940 g.

## DISCUSSION

The mean IVBF determined by the present can also be expressed as half life and appears to normal pregnancies very similar to the value reported by Dixon *et al.* (8) who used the  $^{22}\text{Na}$  method. The flow values measured by  $^{133}\text{Xe}$  *et al.* (6) with their invasive  $^{133}\text{Xe}$  method were than ours, however. Gitsch and Janisch (11) intravenous  $^{99}\text{Tc}^{99\text{m}}$  method reported a value for IVBF almost identical to ours, the difference being that they expressed the flow in ml of placental tissue instead of 100 ml of pool.

The mean IVBF in essential hypertension in this study was  $112\pm39$  ml/100 ml/min ( $p<0.05$ ) in severe pre-eclampsia  $99\pm27$  ( $p<0.001$ ). It is known that in pregnancies complicated by hypertension or pre-eclampsia the IVBF can be seriously reduced. Browne and Veall (3) in their pioneering work found a reduction of 60 per cent in the of patients with essential hypertension. Suonio reported also a significant decrease of tracer accumulation ( $^{99}\text{Tc}^{99\text{m}}$ ) in pre-eclampsia and essential hypertension. In pregnancies complicated by hypertension, proteinuria or edema, elevated uterine (14) and constriction of the supplying arteries result in diminished intervillous space perfusion despite the elevated maternal blood pressure. Late pregnancy morphological abnormalities may become and ultimately dominate the picture. Exclusive vascular lesions in decidual arteries have been shown to be a feature of hypertensive pregnancies (9, 22-24). All these changes may result in a reduction of the placental flow rate.

According to our findings the mean IVBF

abetes mellitus (class B-E) was the lowest (89/100 ml/min). In milder diabetes (class A) the IVBF was of the same order as in essential hypertension or dysmaturity. Gitsch and Janusch (11) also reported a very low IVBF (31 per cent of the normal) for diabetes mellitus. In severe diabetes mellitus the maternal hemodynamic factors and structural changes of the vessels may be grossly altered. The effect of hyperinsulinemia may result in accelerated cell division and an increased placental cell size. This leads to a prolonged proliferation of cytotrophoblast and a failure of their detachment during the second half of pregnancy. The total intervillous space volume may thus be considerably reduced due to edema of the villi (1) which with other changes may lead to altered flow through the intervillous space. It is probable that there is a good correlation between the severity of the disease and the reduction of IVBF. Cholestasis of pregnancy is associated with an elevated perinatal mortality of unknown etiology (10, 13, 23). The mean IVBF in this group was almost as low as in the severe forms of diabetes mellitus (class B-E). No studies of placental hemodynamics in cholestasis of pregnancy can be found in the literature and hence the mean value of IVBF (94/100 ml/min) now recorded cannot be compared with others. In cholestasis of pregnancy however placental permeability for certain amino acids is altered (28). Accordingly there may also be some differences in the diffusion of these into fetal circulation. However this diffusion into the fetal circulation is low even in normal cases and does not appreciably influence the results (17). It is evident that more studies are needed to explain the placental hemodynamics found in cholestasis of pregnancy.

Growth retardation is widely reported as the common consequence of impaired placental blood flow (5, 27). Our results do not strongly support this opinion. The mean flow (109 ml/100 ml/min) in this group was in fact significantly ( $p < 0.01$ ) higher than in the normal group, however it was not as high as that found in the groups with severe pre-eclampsia and diabetes mellitus (class B-E) in which the infants were mostly (in 55-86 per cent) normal in birth weight. Yet both Chatfield (5) and Suonio (25) using accumulation methods found that the placental uptake of tracer in pregnancies resulting in growth retarded babies was significantly reduced. Wolfson and colleagues (27) using an inhalation method ( $^{133}\text{Xe}$ ) also recorded significantly longer half lives in pregnancies

with growth retardation. Crob *et al* (7) used the estimation of the placental blood flow for growth retardation babies to discriminate between a placental and an extraplacental origin of the disorder. It is obvious that many of the dysmature babies in our study are growth retarded because of extraplacental, e.g. genetic causes and this may explain the relatively moderate decrease in the mean IVBF.

Our present findings indicate some new aspects of placental hemodynamics in complicated late pregnancy. A comparison of the results with earlier findings is not easy because of differences in methodology and quantification. The disadvantages and benefits of our method have been discussed elsewhere (20). It can be regarded as a safe method for the fetus and the mother and therefore can be repeated in the follow-up of high risk pregnancies.

Although this method cannot yet be considered a routine clinical tool, it seems to be of great value in the interpretation of the hemodynamic background of some high risk groups in late pregnancy. The reproducibility of this method is about 20 ml/100 ml/min ( $r = 0.88$ ). Detailed flowmetric animal experiments could give more accurate information on the value of this method in determining IVBF quantitatively.

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According to our findings the mean IVBF

## WATER AND ION METABOLISM IN PLACENTA (III)

Net movement of water monovalent and divalent cations in slices from rabbit placenta at different periods of gestation incubated under metabolically favorable conditions

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Rabbit placenta slices incubated at 38 °C in oxygenated medium in the presence of endogenous substrates 5 mM glucose after a period of preincubation of 120 min (0-1 °C) show a net accumulation of potassium and extrusion of water sodium and calcium. The movement of potassium sodium and calcium appears to be related to the rate of oxygen consumption of the slices and to be inversely proportional to the age of the tissue. The efficiency of calcium movement is also dependent on the nature and composition of the incubation medium. In this respect the potassium concentration of the medium plays a major role. The movement of water on the other hand seems to be completely independent from both the rate of respiration and the age of the tissue. The results are discussed in terms of the relation between placenta age and senescence and efficiency of the mechanisms devoted to the cell regulation of water and ions content.

In preceding papers of this series we have presented evidence for the different water and ion composition of placental tissue in relation to its age (2) together with the peculiar changes undergone by the tissue water and ion gradients during incubation under metabolically unfavorable conditions (3). In this paper we show the results of experiments performed with the aim of elucidating from a metabolic point of view the basic mechanisms of regulation of water and ion content in placenta slices incubated *in vitro* in relation to the tissue age and senescence. The results obtained clearly show that placenta slices after a period of incubation at 0-1 °C during which they reach a chemical equilibrium with the incubation medium (3) are indeed capable of regenerating ion gradients across cell membrane and of extruding the excess of water previously gained. Moreover the efficiency of these processes which on the other hand represent the reversible phase of water and cell edema is strictly dependent on

- the nature of the incubating medium. Rabbit plasma in fact appears more suitable in comparison with an artificial saline solution (Ringer Tris).
- The potassium content of the incubation medium. Increasing up to 15 mM the concentration of plasma potassium in fact the recovery of ion gradients is more effective.
- The rate of O<sub>2</sub> consumption of the tissue. This parameter which gives a reliable idea of the energy state of the cell appears to be in turn related both to the age and senescence of placenta and to the incubation conditions.
- The age and senescence of placental tissue. Unlike water extrusion which is almost equally efficient in all the conditions tested the regeneration of ion gradients especially those of sodium and potassium is 100-200 per cent more effective in the younger than in the beyond term placentae. All these results will be discussed as evidence for the decreased effectiveness of basic physiological mechanisms meant to preserve cell homeostasis during placental growth and senescence. Some observations on the proper incubation conditions for placental tissue will also be discussed.

Table 1. Inulin space ( $\alpha$  inulin) in slices from rabbit placenta at different periods of gestation incubated in Ringer Tris or Plasma both at 0-1 °C and 38 °C

Gest period	0-1 °C		38 °C	
	Ringer Tris	Plasma	Ringer Tris	Plasma
II	383 ± 01(16)	266 ± 03(9)	691 ± 02(7)	459 ± 01(9)
IV	363 ± 03 (6)	293 ± 03(6)	n.d.	460 ± 07(6)
V	383 ± 02 (9)	326 ± 05(3)	717 ± 1 (5)	532 ± 04(5)

After equilibration slices inulin content has been determined on the perchloric acid extract following Kulka (Ref no 3)

Table II Total water content and size of intra and extracellular water compartments in slices from rabbit placenta at different periods of gestation incubated under metabolic favourable conditions (38 °C) in different suspending media after 120 min of preincubation at 0–1 °C

A Preincubation 120 min at 0–1 °C

Period of gestation	Ringer Tris			Plasma			Plasma + 10 mM K <sup>+</sup>		
	H <sub>2</sub> O <sub>tot</sub>	H <sub>2</sub> O <sub>i</sub>	H <sub>2</sub> O <sub>e</sub>	H <sub>2</sub> O <sub>tot</sub>	H <sub>2</sub> O <sub>i</sub>	H <sub>2</sub> O <sub>e</sub>	H <sub>2</sub> O <sub>tot</sub>	H <sub>2</sub> O <sub>i</sub>	H <sub>2</sub> O <sub>e</sub>
II	6.8 ± 1 (45)	4.2	2.6	5.3 ± 0.6 (44)	3.7	1.6	5.3 ± 0.7 (8)	3.7	1.6
IV	6.4 ± 1 (18)	4.0	2.4	5.6 ± 0.6 (25)	3.9	1.7	5.7 ± 0.9 (8)	3.9	1.7
V	6.4 ± 1 (15)	4.0	2.4	5.6 ± 0.2 (18)	3.9	1.7	5.8 ± 0.4 (8)	3.9	1.7

\*The size of intra and extracellular water compartments has been calculated on the basis of the space of inulin (mean value at 0–1 °C and 38 °C either in Ringer Tris or Plasma for the different periods of gestation (Table I))

B Incubation 60 min at 38 °C after the preincubation

Period of gestation	Ringer Tris			Plasma			Plasma + 10 mM K <sup>+</sup>		
	H <sub>2</sub> O <sub>tot</sub>	H <sub>2</sub> O <sub>i</sub>	H <sub>2</sub> O <sub>e</sub>	H <sub>2</sub> O <sub>tot</sub>	H <sub>2</sub> O <sub>i</sub>	H <sub>2</sub> O <sub>e</sub>	H <sub>2</sub> O <sub>tot</sub>	H <sub>2</sub> O <sub>i</sub>	H <sub>2</sub> O <sub>e</sub>
II	5.8 ± 1 (26)	1.7	4.1	4.4 ± 0.2 (44)	2.3	2.1	4.1 ± 0.1 (8)	2.1	2.0
IV	5.5 ± 0.9 (18)	1.6	3.9	4.6 ± 0.6 (25)	2.4	2.2	4.6 ± 0.3 (8)	2.4	2.2
V	5.3 ± 2 (12)	1.6	3.9	4.4 ± 0.6 (18)	2.3	2.1	4.6 ± 0.1 (8)	2.4	2.2

## MATERIAL AND METHODS

The methods followed in the present work are essentially the same as those described in the preceding papers (2, 3).

Rabbit pregnancy was arbitrarily subdivided into the usual five periods (2) in this work however we restricted the observation to three periods only: period II (16th–20th day of gestation), period IV (26th–30th day of gestation) and period V (31st–33rd day of gestation). To bring the pregnancy to the period V animals were treated with HCG as already described (2). Placental slices cut free hand by razor blade were kept for 120 min at 0–1 °C in an ice bath and suspended separately in each of the following media: 1) Ringer Tris (158 mM Na<sup>+</sup>, 5 mM K<sup>+</sup>, 5 mM Ca<sup>2+</sup>, 1.0 mM Mg<sup>2+</sup>, 1.0 mM SO<sub>4</sub><sup>2-</sup>, 175.6 mM Cl<sup>-</sup> and 10 mM Tris HCl pH 7.4); 2) rabbit plasma with the addition of 10 mM Tris HCl pH 7.4; 3) rabbit plasma with the addition of 10 mM K<sup>+</sup> (KCl) and 10 mM Tris HCl pH 7.4. Rabbit plasma was obtained from the same pregnant animals by collecting the blood before the placenta was removed.

After the cold preincubation the slices were transferred to Warburg vessels with a central well and incubated for 1 hour at 38 °C in the presence of 5 mM glucose (added only to the artificial Ringer Tris) and of pure oxygen as gas phase. During this period the O<sub>2</sub> uptake was determined by the Warburg method in the presence of 0.2 ml of 20 per cent KOH in the central well. At the end of the incubation at 38 °C the slices were carefully withdrawn, gently blotted on hardened filter paper and placed in tared plastic screw cup bottles. The wet and dry weight of the tissue and the ion content of the slices were determined as described before (2). In parallel experiments the space of inulin of the slice was determined performing the incubation in the above-

mentioned media in the presence of 0.5 per cent previously described (3). Sodium potassium calc magnesium were determined by atomic absorption spectrophotometry. Deionized water (>20 mΩ) was throughout the work.

## RESULTS

The inulin space ( $\alpha$  inulin) determined in slices of rabbit placenta at different periods of gestation incubated 120 min at 0–1 °C and subsequently at 38 °C both in Ringer Tris and plasma is shown in Table I. The  $\alpha$  inulin, which gives a measure of extracellular space, is not significantly different in slices from the three gestation periods studied also ref (3) but its mean value is about 25 per cent lower in the tissue suspended in plasma. This is probably due to the different oncotic pressure of suspending media. A substantial increase of  $\alpha$  inulin of about 70 per cent can be observed in media when the slices are transferred from 0–1 °C to 38 °C. This indicates that a large change in distribution of water between intra and extracellular compartments when active metabolic conditions are restored occurs.

Total tissue water content decreases in all the conditions, about 15–20 per cent, regardless of both the incubation medium used and the period of gestation (Table II).

### III A $\text{Na}^+$ (mmol/kg d wt) movements in rabbit placenta slices at different periods of gestation in at 0-1 C and 38 C in Ringer Tris Plasma and 10 mM $\text{K}^+$ supplemented Plasma Effect of 1 mM

of ation	0-1 C			38 C	
	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Ringer Tris	Plasma
II	985.4 ± 21.8 (45)	886.0 ± 15.6 (44)	787.4 ± 21.3 (8)	959.4 ± 45.5 (17)	687.0 ± 34.4 (25)
IV	1044.0 ± 57.4 (18)	894.5 ± 14.6 (25)	804.0 ± 39.1 (8)	934.8 ± 70.9 (18)	719.4 ± 25.7 (25)
V	945.5 ± 28.0 (15)	842.5 ± 24.6 (18)	837.5 ± 28.6 (8)	897.0 ± 39.0 (12)	786.8 ± 19.7 (18)

#### Table III A continuation

C (cont.)		Per cent recovery			
Plasma + 10 mM $\text{K}^+$	Plasma + 1 mM ouabain	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Plasma + ouabain
7 ± 22.2 (8)	897.6 ± 38.4 (4)	4.3	39.0	68.6	~ 2.3
13 ± 28.4 (8)	876.3 ± 41.7 (4)	20.0	44.1	61.5	4.6
14 ± 34.2 (8)	890.0 ± 13.6 (4)	11.0	16.5	30.1	~ 14.0

The net extrusion of water from the slices together with the swelling of the interstitial compartment which take place at 38°C cause a drastic shrinkage of the intracellular compartment. From the data of Table II in fact it can be seen that at 38°C placental slices lower their water content by 40-60 per cent. The expulsion of water takes place in all the suspension media. The recovery can be considered complete. The  $\text{H}_2\text{O}_1$  in some cases is even lower than in fresh tissue (2).

Table III summarizes the content of  $\text{Na}^+$  and  $\text{K}^+$  in the placenta slices at different periods of gesta-

tion incubated in Ringer Tris plasma and plasma + 10 mM  $\text{K}^+$  for 60 min at 38°C after the preincubation of 120 min at 0-1°C.

In the last columns of the table the per cent recovery in each condition tested is also reported. The slices incubated at 38°C in Ringer Tris expel and take up a small amount of  $\text{Na}^+$  and  $\text{K}^+$  respectively regardless of the period of gestation. The per cent recovery in fact is only in this case in the range of 5-20 per cent.

The activity can be stimulated by incubating the slices in rabbit plasma.  $\text{Na}^+$  extrusion in fact in

### Table III B $\text{K}^+$ (mmol/kg d wt) movements in rabbit placenta slices at different periods of gestation in at 0-1 C and 38 C in Ringer Tris Plasma and 10 mM $\text{K}^+$ supplemented Plasma Effect of 1 mM

of ation	0-1 C			38 C	
	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Ringer Tris	Plasma
I	184.8 ± 7.4 (45)	203.8 ± 11.0 (44)	197.5 ± 18.7 (8)	191.7 ± 11.8 (17)	261.6 ± 7.4 (25)
II	144.0 ± 6.1 (18)	127.4 ± 4.8 (25)	153.5 ± 11.1 (8)	159.1 ± 5.5 (18)	152.6 ± 3.7 (25)
III	133.2 ± 12.6 (15)	143.2 ± 7.9 (18)	138.5 ± 21.4 (8)	142.9 ± 11.2 (12)	136.2 ± 5.2 (18)

#### Table III B continuation

(cont.)		Per cent recovery			
Plasma + 10 mM $\text{K}^+$	Plasma + 1 mM ouabain	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Plasma + ouabain
± 34.3 (8)	103.0 ± 9.3 (4)	2.0	18.2	64.8	~ 31.7
± 24.5 (8)	59.3 ± 4.2 (4)	5.3	8.2	42.1	22.4
± 27.2 (8)	97.4 ± 4.5 (4)	4.1	3.1	19.9	~ 20.4

Per cent recovery has been calculated on the basis of the potassium content of the fresh tissue taken from Ref. no. 2

Table IV A Sodium [ $\text{Na}^+$ , (mM)] intracellular concentration of rabbit placenta slices at different periods of gestation incubated at 0–1 °C and 38 °C in different suspending media

Period of gestation	0–1 °C			38 °C		
	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$
II	136.8	171.1	144.5	183.3	154.4	200.9
IV	166.2	160.5	137.3	199.2	154.9	111.5
V	141.6	147.2	145.9	175.5	197.8	162.4

creases to about 40 per cent in the slices of the period II and IV while it is still low (16.5 per cent) in those of period V. As far as  $\text{K}^+$  is concerned the recovery is more pronounced and it also occurs in inverse relation to the age of placenta.

These results show that placenta behaves quite differently from other tissues. The extensive studies of van Rossum (4–8) indicate that slices from rat liver or from some neoplastic tissues are able to regain in the  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  gradients when they are incubated at 38 °C under suitable metabolic condition even when suspended in artificial saline solution.

Preliminary experiments which showed that placenta slices incubated at 38 °C in Ringer Tris but without the previous incubation at 0–1 °C fail to regain ion gradients (results not shown) suggest that the cold incubation does not irreversibly spoil the tissue.

We thought therefore that the discrepancy between liver and placenta might be related to some peculiar constituent of the incubating medium necessary for the placental tissue to keep *in vitro* the ion gradients exhibited *in vivo* (2).

Following this reasoning and knowing that (a) in some systems membrane  $\text{Na}^+$ – $\text{K}^+$  ATPase is strongly dependent on the [ $\text{K}^+$ ] outside the cell (see for review 6) and (b) [ $\text{K}^+$ ] in the fetal blood is higher than that in maternal blood (for review see 1) we increased the [ $\text{K}^+$ ] of the rabbit plasma to 15 mM in an attempt to improve the active ion movements.

Indeed we have found that placenta slices the most active  $\text{Na}^+$  extrusion and  $\text{K}^+$  they are incubated in rabbit plasma with added 15 mM  $\text{K}^+$ . The recovery of the sodium now becomes of 61.5 and 30.1 per cent in periods II, IV and V respectively while that potassium increases to 64.8, 42.1 and 19.9 per cent in the three periods respectively.

It is apparent that the placentae from the term pregnancies always show the lowest recovery. The regeneration of  $\text{Na}^+$  and  $\text{K}^+$  gradients across the plasma membrane implies expenditure of energy utilized by a specific ATPase. This enzyme is known to be sensitive to the inhibition which indeed inhibits also the placenta stimulated ATPase. In the presence of 1 mM ouabain  $\text{Na}^+$  in fact remains high and potassium and the recovery is completely lacking (negative) (Table III). From the knowledge of the intracellular water compartment and the tissue sodium and potassium it is possible to calculate the intracellular concentration of  $\text{Na}^+$ . Such data are reported in Table IV. After incubation in the cold the concentration on the side of the placental cells is equal to that of the medium (see also ref 3). A concentration of 15 mM is found in the slices incubated both in Ringer Tris and plasma or plasma + 10 mM  $\text{K}^+$ . At 38 °C despite the apparent recovery shown in Table III the intracellular concentration of  $\text{Na}^+$  is still low in Ringer Tris and in plasma. Only when the slices

Table IV B Potassium [ $\text{K}^+$ , (mM)] intracellular concentration of rabbit placenta slices at different periods of gestation incubated at 0–1 °C and 38 °C in different suspending media

Period of gestation	0–1 °C			38 °C		
	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$
II	40.9	52.9	46.9	100.7	109.2	179.8
IV	33.0	30.5	39.3	87.3	59.0	99.0
V	30.3	34.5	29.0	77.1	44.7	63.0

Table V A Magnesium [ $Mg^{2+}$  (mmol/kg d wt)] content of rabbit placenta slices at different periods of gestation incubated 120 min at 0–1 °C and 60 min at 38 °C in different oxygenated suspending media

Period of gestation	0–1 °C			38 °C		
	Ringer Tris	Plasma	Plasma + 10 mM K <sup>+</sup>	Ringer Tris	Plasma	Plasma + 10 mM K <sup>+</sup>
II	40.5 ± 1.2 (45)	36.6 ± 1.1 (44)	35.7 ± 2.2 (8)	34.7 ± 1.2 (26)	31.4 ± 1.1 (27)	37.4 ± 2.4 (8)
IV	36.7 ± 0.8 (18)	32.2 ± 0.6 (25)	31.1 ± 1.4 (8)	30.9 ± 0.7 (18)	28.7 ± 0.5 (25)	34.9 ± 1.8 (8)
V	34.8 ± 1.6 (15)	34.5 ± 0.9 (18)	32.4 ± 2.7 (8)	33.0 ± 1.4 (12)	29.2 ± 0.6 (18)	30.4 ± 3.6 (8)

Mean ± SEM (n of observations). For further details see the text.

incubated in the presence of plasma with added 10 mM K<sup>+</sup> (15 mM final) does Na<sup>+</sup> intracellular concentration decrease to about 90 mM in the period II and to about 100 mM in the period IV while it is still high in the slices of the period V.

An actual recovery is therefore present only in the slices of the periods II and IV whereas it is absent in the beyond term placenta. It must be noted that some of the [Na<sup>+</sup>]<sub>i</sub> calculated in Table IV exceed the [Na<sup>+</sup>]<sub>o</sub> because of experimental error. The calculated intracellular concentration of K<sup>+</sup> behaves in a way similar to Na<sup>+</sup>. The concentration in fact higher in the K<sup>+</sup> supplemented plasma and the uptake at 38 °C is greater in the younger placenta. In Table V the results of experiments are reported which concern the net movement of Mg<sup>2+</sup> and Ca<sup>2+</sup>. The content of Mg<sup>2+</sup> does not seem to show significant variation between the various conditions tested. The amount of this bivalent cation is constant at a value of about 30–40 mmol/kg d wt with small variation of no statistical significance and unrelated to the design of the experiment.

In contrast Ca<sup>2+</sup> behaves quite differently from K<sup>+</sup>. At 0–1 °C in fact Ca<sup>2+</sup> enters the tissue and increases significantly in comparison with fresh tissue. When the slices are transferred to 38 °C a net movement of Ca<sup>2+</sup> is evident. This happens especially in the slices suspended in the K<sup>+</sup> supplemented plasma. Ca<sup>2+</sup> content in fact decreases significantly in the slices of period II and IV to values which are very similar to those of the fresh tissue (2). The efficiency of the expulsion decreases from 60–90 per cent to 30 per cent in the beyond term placenta. In incubation in Ringer Tris and plasma alone the Ca<sup>2+</sup> in the tissue at 38 °C increases to values higher than at 0–1 °C.

On the basis of the results shown up to now we looked for a possible correlation between the metabolic condition of placenta slices incubated at

38 °C and both the age of the tissue and the incubation medium. To this purpose we measured the O<sub>2</sub> consumption of the slices from placenta at different periods of gestation incubated at 38 °C after cold preincubation in the three different media. Under certain circumstances respiration can be considered as good parameter for evaluating cell energy metabolism.

The results are reported in the last Table (VI). The rate of oxygen uptake is primarily dependent on the age of the tissue as had been previously shown on different placental systems (5–7) but it is also strongly related to the nature and composition of the incubating medium. Respiration in fact decreases significantly by 40–60 per cent in the period IV and V irrespective of the suspension medium. On the other hand O<sub>2</sub> consumption increases by 50 and 70 per cent in plasma and K<sup>+</sup> supplemented plasma respectively. The increase is less pronounced in the placenta of period V (about 5 per cent). The addition of ouabain inhibits O<sub>2</sub> uptake uniformly by 15 per cent.

## DISCUSSION

**Water movement at 38 °C.** The results presented in the previous section show clearly that placenta cells are able to extrude under the appropriate metabolic conditions a substantial amount of water taken up during the period of incubation at 0–1 °C. The decrease of the total tissue water is rather small (14–20 per cent) but is accompanied by a more substantial shrinkage of the intracellular compartment. This compartment in fact is reduced in size by about 30–60 per cent. Although the expulsion of water from placental tissue is dependent on the metabolic state of the cells (it takes place at 38 °C) it does not seem to be related to the rate of oxygen consumption and the age of the tissue. The expulsion oc-



Table IV A Sodium [ $\text{Na}^+$ , (mM)] intracellular concentration of rabbit placenta slices at different periods of gestation incubated at 0–1 °C and 38 °C in different suspending media

Period of gestation	0–1 °C			38 °C		
	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$
II	136.8	171.1	144.5	183.3	154.4	89.9
IV	166.2	160.5	137.3	199.2	154.5	111.5
V	141.6	147.2	145.9	175.5	197.8	162.4

creases to about 40 per cent in the slices of the period II and IV while it is still low (16.5 per cent) in those of period V. As far as  $\text{K}^+$  is concerned the recovery is more pronounced and it also occurs in inverse relation to the age of placenta.

These results show that placenta behaves quite differently from other tissues. The extensive studies of van Rossum (4–8) indicate that slices from rat liver or from some neoplastic tissues are able to regain in the  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  gradients when they are incubated at 38 °C under suitable metabolic condition even when suspended in artificial saline solution.

Preliminary experiments which showed that placenta slices incubated at 38 °C in Ringer Tris but without the previous incubation at 0–1 °C fail to regain ion gradients (results not shown) suggest that the cold incubation does not irreversibly spoil the tissue.

We thought therefore that the discrepancy between liver and placenta might be related to some peculiar constituent of the incubating medium necessary for the placental tissue to keep *in vitro* the ion gradients exhibited *in vivo* (2).

Following this reasoning and knowing that (a) in some systems membrane  $\text{Na}^+$   $\text{K}^+$  ATPase is strongly dependent on the  $[\text{K}^+]$  outside the cell (see for review 6) and

(b)  $[\text{K}^+]$  in the fetal blood is higher than that in maternal blood (for review see 1) we increased the  $[\text{K}^+]$  of the rabbit plasma to 15 mM in an attempt to improve the active ion movements.

Indeed we have found that placenta slices the most active  $\text{Na}^+$  extrusion and  $\text{K}^+$  uptake are they are incubated in rabbit plasma with added mM  $\text{K}^+$ . The recovery of the sodium now becomes of 58.6, 61.5 and 30.1 per cent periods II, IV and V respectively while that potassium increases to 64.8, 42.1 and 19.9 per cent the three periods respectively.

It is apparent that the placentae from the term pregnancies always show the lowest. The regeneration of  $\text{Na}^+$  and  $\text{K}^+$  depends across the plasma membrane implies expenditure of energy utilized by a specific ATPase enzyme is known to be sensitive to the inhibitor which indeed inhibits also the placental stimulated ATPase. In the presence of 1 mM slices  $\text{Na}^+$  in fact remains high and potassium and the recovery is completely lacking (negative) (Table III). From the knowledge of intracellular water compartment and the tissue sodium and potassium it is possible to calculate the intracellular concentration of these. Such data are reported in Table IV. After incubation in the cold the concentration on the inside the placental cells is equal to that of the medium (see also ref 3). A concentration of about 150 mM is found in the slices incubated both in Tris and plasma or plasma + 10 mM  $\text{K}^+$ . At 38 °C despite the apparent recovery shown in Table I intracellular concentration of  $\text{Na}^+$  is still high in Ringer Tris and in plasma. Only when the

Table IV B Potassium [ $\text{K}^+$ , (mM)] intracellular concentration of rabbit placenta slices at different periods of gestation incubated at 0–1 °C and 38 °C in different suspending media

Period of gestation	0–1 °C			38 °C		
	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$	Ringer Tris	Plasma	Plasma + 10 mM $\text{K}^+$
II	40.9	52.9	46.9	100.7	109.2	179.8
IV	33.0	30.5	39.3	87.3	59.0	99.0
V	30.3	34.5	29.0	77.1	54.7	63.0

We have previously shown that placental slices incubated under metabolically unfavorable conditions (0–1 °C) equilibrate their ion content with the medium regardless of the period of pregnancy.

In contrast the recovery at 38 °C appears to be strongly dependent on the degree of maturity or of the tissue. The period II placentae are able to recover almost completely their  $\text{Na}^+$  and  $\text{K}^+$  contents with an efficiency of about 65 per cent (see Table II).

The efficiency declines to about 50 per cent in term (period IV) whereas it further decreases in placentae brought beyond term. In the latter case the decrease in the  $\text{Na}^+$  and the increase in the  $\text{K}^+$  content of the slices (Table III) the calculated intracellular concentration shows that  $\text{Na}^+$  movement is only apparent while that of  $\text{K}^+$  is rigorous (Table IV). In beyond term placentae the decrease of tissue  $\text{Na}^+$  is than not linked to active

passive transfer dependent on water extrusion. The relation between the active movement of ions and tissue metabolic state is proved by the data concerning the respiration of placental slices incubated in various media at different periods of gestation. It is apparent that the higher the rate of  $\text{O}_2$  consumption the more efficient the ion translocation. Placental slice  $\text{O}_2$  uptake is in turn dependent on both tissue age and the incubation medium. At 38 °C magnesium does not show significant changes further proving that this ion is one of the most stable in the cell. It is probably mostly bound to intracellular structures as in other systems.

The behaviour of  $\text{Ca}^{2+}$  is rather different. It is during the cold incubation and is actively extruded under metabolically favorable conditions. The efficiency of the extrusion is high (60–90 per cent) in period II and IV whereas it declines to 30 per cent in the beyond term placentae. There is an apparent discrepancy between the data concerning  $\text{Ca}^{2+}$  movement in this study compared with those of our previous work (3). We increased the concentration of  $\text{Ca}^{2+}$  in the medium in these experiments to a more physiological level (5 mM). A higher  $\text{Ca}^{2+}$  content in the external medium could increase cell membrane stability and affect its permeability to other ion species ( $\text{Na}^+$  and  $\text{K}^+$ ).

We wish to emphasize the influence of the composition of the incubating medium on placenta tissue functions. We have seen that plasma and the further addition of 10 mM  $\text{K}^+$  to plasma improves both the extrusion and ion translocation in placentae. In particular the  $\text{K}^+$  supplemented plasma is the only

medium in which a real gain of ion gradients ( $\text{Na}^+$  and  $\text{K}^+$ ) takes place and it is the only condition in which net extrusion of  $\text{Ca}^{2+}$  is evident. These observations are of general interest in improving placental performances during *in vitro* studies.

## CONCLUSION

The results obtained in the present work lead us to the following conclusions. Rabbit placenta slices incubated under metabolically favorable conditions after a period of preincubation at 0–1 °C are able to bring about the net extrusion of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  and the uptake of  $\text{K}^+$ . The active movement of ions is strictly dependent on the incubation conditions, the presence of high  $[\text{K}^+]$  being essential. Further  $\text{Na}^+$  and  $\text{Ca}^{2+}$  extrusion and  $\text{K}^+$  uptake take place more efficiently in younger placentae decreasing toward the end of pregnancy and disappearing in the tissue pharmacologically brought beyond physiological term. The net ion movement is finally tightly coupled to the cell energy supply. We have demonstrated that a direct relationship exists between the  $\text{O}_2$  consumption rate and ion movement. Tissue oxygen uptake is in turn influenced by the incubation conditions and tissue age. Finally placental slices incubated in oxygenated medium at 38 °C extrude the excess water taken up during its stay at 0–1 °C by metabolism-dependent mechanisms. The water extrusion, unlike the recovery of ion gradients, takes place with equal efficiency regardless of the nature of the incubation medium and of the age of the tissue.

Further studies are in progress to better define the source of the energy necessary for the maintenance of ion gradients and the biophysical characteristics of the process.

## ACKNOWLEDGEMENTS

This work has been supported by Grants from CNR (n. 76 00282, 76 01520 04) and Ministero della Pubblica Istruzione of Italy. We thank Dr. G. Carelli and the Institute of Occupational Medicine of this University for making available the atomic absorption facility.

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*Submitted for publication October 26 1977*

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## A LONGTERM ISOTOPE RENOGRAPHY FOLLOW UP OF PATIENTS FOLLOWING PREGNANCY COMPLICATED BY PRE ECLAMPSIA AND HYPERTENSION

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**Abstract** 61 patients were studied with the renography and urography during puerperium ten years ago and now again. The technique and analyses were identical. The results showed that some of these parameters (SV, K, and T 1/2) were either decreased or prolonged more than ten per cent during this period in nearly all cases. However, these changes occurred in both the normal and complicated groups without any correlation with the original diagnosis from the first study. Hypertension has developed in 10 per cent of the toxemic group.

Whether pre eclampsia or hypertension during pregnancy causes permanent changes in the maternal kidneys has been a problem which has occupied many research workers. In most cases the changes have disappeared during the first few months after delivery (1, 2, 9). In Friedberg's study (5) 15 per cent of toxemic patients had renal lesions after delivery and 50 per cent were reported in Dieckman's study (4). In other studies follow up was limited to a few months post partum. In this study renographic findings obtained ten years ago (6) were compared with the current findings.

## MATERIAL AND METHODS

The present series consisted of previously examined patients: 1 patients after uncomplicated pregnancy, 78 after toxemia and 7 after hypertensive pregnancy (Table 1) who were invited for a control examination. The invitation was

accepted by 61 mothers. Of these mothers 16 had had an uncomplicated pregnancy, 30 mild and 8 severe pre-eclampsia and 7 chronic hypertension as a complication present in the former examination. All patients underwent isotope renography and in 25 cases intravenous pyelography was performed.

**Analysis of the renogram.** The comparison of previous post partum renograms with control renograms was made in accordance with the original analysis, i.e. (6) using as parameters: the secretion value (=SV), the accumulation peak (=K) and the half time of the excretion phase (=T 1/2), the main interest being directed towards changes in SV of over 10 per cent.

The empirical normal range of the parameters was: SV = 1-25, the culmination peak = 6 minutes and the half time of the excretion phase = 18 minutes. The SV value represents the tubular capacity to receive the tracer, the accumulation peak reflects the cellular excretion capacity and T 1/2 mainly the renal excretion of the tracer.

## RESULTS

**Mothers without the complication of hypertension in pregnancy (controls).** The changes in SV values are shown in Table 2. SV was increased in 31 per cent of the cases when compared with the previous examination, totally decreased in 60 per cent, in 50 per cent on the left side, 69 per cent on the right side and bilaterally in 50 per cent. Of three patients in whom the SV values had decreased bilaterally more than 10 per cent, one had contracted rheumatoid arthritis.

Table 1. The distribution of the maternal according to the first study

Diagnosis	The first study	The second study	Between the studies developed a chronic disease	
	Cases	Cases	Cases	DGN
Normal pregnancy	27	16	1	arthritis
Mild pre-eclampsia	48	30	5	hypertension
Severe pre-eclampsia	30	8	2	hypertension
Hypertension	7	7	7	hypertension
Total	112	61	15	

Table II *The SV change in the control group the pre eclampsia group and the hypertension group*

	Control			Mild pre-eclampsia			Severe pre-eclampsia			Hypertension		
	Left	Right	Both	Left	Right	Both	Left	Right	Both	Left	Right	Both
Unchanged	3 (19)			3 (10)	6 (20)	2	1 (12)					
Increased	5 (31)	5 (31)	5	8 (27)	5 (17)	3	1 (12)	1 (12)	1	1 (14)		
Decreased	8 (50)	11 (69)	8	19 (63)	19 (63)	17	6 (75)	7 (88)	6	6 (85)	7 (100)	6
Change												
> 10 per cent	5 (31)	9 (56)	3	10 (33)	13 (43)	6	1 (12)	1 (12)		4 (57)	7 (100)	4
Cases no	16	16		30	30		8	8		7	7	

Both = The simultaneous change in both kidneys

The figures within parenthesis denote number in per cent

The culmination peak (Table 3) had remained unchanged in 62 per cent increased in 28 per cent and decreased in only 9 per cent of the control patients

The half time of the excretion phase showed no change in 25 per cent it was shortened in 44 per cent and prolonged in 19 per cent of the cases T 1/2 was impossible to analyse in two renograms (Table 4)

Eight mothers had undergone intravenous pyelography both in 1967 and the present time In one case with a previously normal result hydronephrosis had developed in this patient the above parameters were also decreased The urogram of the rheumatoid patient had been abnormal at the first examination

**Mild pre-eclampsia** The SV value was unchanged bilaterally in 7 per cent of the cases increased in 10 per cent and decreased in 57 per cent There was a decrease in both kidneys in 63 per cent of subjects Five patients had become hypertensive and in all these cases the SV value was decreased on both side

The culmination peak was unchanged bilaterally in 50 per cent of the cases shortened in only one and prolonged in 23 per cent

The half time of the excretion phase was unchanged bilaterally in 17 per cent both shortened in 17 per cent and prolonged also in 17 per cent of the cases

In the cases which had become hypertensive the 1/2 was also prolonged with a simultaneously increased SV value

In this group intravenous pyelography was done in 9 cases of which seven were interpreted as normal one case a picture of chronic pyelitis was found another case of the same kind of changes had already been observed ten years previously In both cases SV values were decreased by 18 per cent

**Severe pre eclampsia** The SV value was increased bilaterally in one case decreased in six cases change being over 10 per cent in two cases The culmination time was unchanged bilaterally in cases (38 per cent) decreased in three cases (12 per cent) and increased unilaterally in two cases (24 per cent)

The half time of the excretion phase was unchanged bilaterally in two cases shortened in two cases prolonged in one case in one case an unilateral shortening in the T 1/2 was observed Intravenous pyelography done in two cases showed no pathological changes irrespective of the fact that the other patients became chronic hypertensive

**Chronic hypertension** The patients in this group received hypotensive medication With the exception

Table III *The culmination peak in the control group the pre eclampsia group and the hypertension group*

	Control			Mild pre-eclampsia			Severe pre eclampsia			Hypertension		
	Left	Right	Both	Left	Right	Both	Left	Right	Both	Left	Right	Both
Unchanged	10 (62)	10 (62)	7	20 (62)	18 (60)	15	3 (38)	3 (38)	3	2 (29)	3 (43)	2
Fast	4 (25)	5 (31)	3	3 (10)	3 (10)	1	2 (24)	2 (24)				
Indefinable					2 (7)							
Prolonged	2 (13)	1 (7)	1	7 (28)	7 (23)	5	3 (38)	3 (38)	3	5 (71)	4 (57)	4

Both = The simultaneous change in both kidneys

The figures within parenthesis denote number in per cent

Table IV The half time of the excretion in different groups

	Control			Mild pre-eclampsia			Severe pre-eclampsia			Hypertension		
	Left	Right	Both	Left	Right	Both	Left	Right	Both	Left	Right	Both
Unchanged	4 (25)	4 (25)	3	8 (27)	7 (23)	5	3 (37)	2 (25)	2	2 (27)	4 (57)	3
Fast	8 (50)	6 (37)	4	7 (23)	6 (20)	5	2 (25)	3 (37)	2	2 (27)		
Indefinable	1 (6)	3 (19)	1	7 (23)	9 (30)	7	2 (25)	2 (25)	2	1 (19)		
Prolonged	3 (19)	3 (19)	1	8 (27)	8 (27)	5	1 (13)	1 (13)	1	2 (27)	3 (43)	1

h = The simultaneous change in both kidneys

Figures within parenthesis denote number in per cent

one patient the renogram on the left kidney showed that the SV value was decreased by more than 10 per cent in all cases and that this was bilateral in four

The SV value of the right kidney was decreased more than 10 per cent in seven of the cases

The culmination time was unchanged in two cases and prolonged in four cases

The half time of the excretion phase was unchanged in three cases bilaterally and prolonged in only one case

Six patients had undergone repeated intravenous urography. In two cases pathological changes had already been observed in the previous examination. In the examination two more cases with right-sided renal changes were found.

## DISCUSSION

During ten years 1963-1964 when the first part of this study took place renography was in the first stages of development. Nowadays it has reached a permanent status as a screening test of tubular function. Nevertheless the mathematical interpretation of the renogram still remains a problem. That is why in this study the old empirical quantification developed in Heidelberg was used being independent of each other prevents the possible errors in one value from affecting the others. In practice it has become evident that the SV value is the most informative of the parameters. It is impossible to estimate the exact effect of physiological ageing nevertheless in this study a ten per cent change in the values occurred during the 10 years between the examinations. In 18 per cent (7 cases) of our toxemic patients had developed chronic hypertension. This result is similar to the studies previously mentioned. A decrease in the SV values had taken place in 68 per cent (83 kidneys) of the total study patients and a greater than ten per

cent change in 41 per cent (50 kidneys). 44 per cent of the control group, 38 per cent of the mild pre-eclampsia group, 12 per cent of the severe pre-eclampsia group and 78 per cent of the chronic hypertension group. The decrease was bilateral in 37 patients (61 per cent) and both bilateral and greater than ten per cent in 13 cases (21 per cent).

The low level of the SV of the right kidney is a phenomenon already observed during pregnancy. In this study there was a decrease of more than ten per cent on the left side in 33 per cent (20 cases) and on the right side in 49 per cent (30 cases).

The culmination peak time was also prolonged. This prolongation can be explained by the decrease in the capacity or affinity of the tubular cells which is probably related to physiological ageing.

The time of the excretion phase reflects the speed of the disappearance of the tracer from the measuring field. In renograms performed during the puerperium the dilatation of the renal pelvis and the ureter have the effect of prolonging the T 1/2 which makes the comparison between our series unreliable. Omitting chronic hypertension the state of health of the patient during the previous pregnancy did not correlate with the present renal function. Nevertheless in only nine cases did any of the measured parameters remain unchanged or improve. In epidemiological studies in the United States a clear correlation between previous toxemia and secondary hypertensive disease has been shown (8). We must not forget too that a hypertensive family history and obesity are correlated with the existence of chronic hypertension (3). These latter facts may cause difficulties with interpretation of our results. Of the parameters used in this study the SV has been shown to be most informative in practice. Also in this comparative study the changes in these parameters have told us about hidden renal lesions or overstrain caused by pregnancy and its complications.

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Submitted for publication July 27 1978

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# PROGESTERONE AND HUMAN CHORIONIC GONADOTROPHIN IN SERUM AND PREGNANDIOL IN URINE IN THREATENED ABORTION

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**Abstract** Progesterone and human chorionic gonadotrophin (HCG) in serum and pregnandiol in urine were measured in 64 patients admitted to hospital because of threatened abortion. Blood samples were taken and urine specimens collected at regular intervals during admission and after discharge during the rest of the pregnancy. A reference range was worked out for each hormone based on the hormone values obtained from the pregnancies proceeding to term. The predictive significance of values within and below the reference range was determined for the initial sample and for serial samples. An association between hormone levels and outcome of pregnancy was observed but it is concluded that both single and serial determinations of progesterone and pregnandiol and serial determinations of HCG are unsatisfactory for the evaluation of threatened abortion. However an initial progesterone value below the reference range and HCG values below 10 000 mIU/ml before the 8th and 15th week of pregnancy was in every case followed by spontaneous abortion. A hormonal test of placental origin is recommended for monitoring threatened abortion.

Human chorionic gonadotrophin (HCG) is produced by the syncytiotrophoblast in the placenta (8). For many years the detection of HCG in urine and serum has been used to diagnose and evaluate early pregnancy. The corpus luteum seems to be the main site of progesterone synthesis up to the 9th to 11th week of pregnancy thereafter the placenta is the main site of production (4). Progesterone is mainly metabolized in the liver and about 10-15 per cent is excreted in urine as pregnandiol (6). The estimation of these hormones is a possible way of monitoring early complicated pregnancy. The value of progesterone, pregnandiol and HCG in evaluation of the prognosis in threatened abortion has previously been investigated with conflicting results (1, 2, 7, 10). The purpose of the present study was to estimate the prognostic value of both single and serial determinations of these three hormones in patients with threatened abortion.

## MATERIAL AND METHODS

This study comprised 72 pregnant women consecutively admitted to a gynecologic department because of vaginal bleeding with or without lower abdominal pain from the 5th to 20th week of pregnancy. Eight patients were excluded for various reasons. This left 64 patients in the study. They have been described in a previous paper (3).

Blood samples for progesterone and HCG determination were taken shortly after admission and daily for the first five days and then twice weekly while in hospital. After discharge samples were taken at least once a month until end of pregnancy. Progesterone was measured in all patients. HCG in all but one. Twenty-four hour urinary pregnandiol excretion was measured in 50 patients. The 24-hour urine specimens were collected twice weekly during admission and after discharge at least once a month until delivery. The results of the hormone determinations did not influence the treatment of the individual patient.

Progesterone in serum was extracted with petroleum ether (bp 40-60 °C) and estimated by radioimmunoassay with an antiserum raised against 11 alpha hydroxyprogesterone coupled with bovine serum albumin. Pregnanediol in urine was measured with a modification of the method described by Kirschner & Lipsett (5). HCG radioimmunoassay was carried out by the double antibody technique as described by Odell *et al.* (11). The HCG antiserum used cross-reacts with luteinizing hormone (LH) but the HCG level in serum samples has never been so low that LH cross reaction will influence the assay.

## RESULTS

Thirty-five (55 per cent) of the 64 patients continued pregnancy until at least the 28th week of gestation. Twenty-nine patients (45 per cent) aborted spontaneously. As previously described (3) a 95 per cent reference range was calculated from the hormone values obtained from the pregnancies proceeding to term. The reference ranges are indicated on the figures. All patients who aborted are indicated on the figures. Single values are marked with a filled circle. In cases where more than one determination was



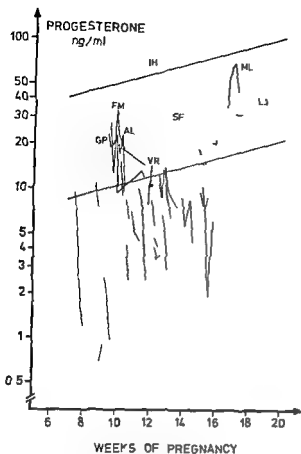


Fig 1 Progesterone in serum in threatened abortion  
 — Pregnancies proceeding to delivery  
 - - - Pregnancies ending in abortion  
 Single values from pregnancies ending in abortion. The 95 per cent reference range is indicated

made the values are connected with solid lines. Values from pregnancies ending in delivery at term are only indicated if any value from the first five days was below the lower limit of the reference range. They are connected with broken lines. Values are described as low if they fall below the lower limit of the reference range and normal when within the reference range.

The results of the progesterone determinations are presented on Fig 1. Only 18 of the 29 patients who aborted had low values at the first blood sample. The first value thus erroneously predicted favorable outcome of the pregnancy in 11 patients who later aborted spontaneously. After serial determinations progesterone levels were low in 23 of the patients that subsequently aborted. Two patients (GP, FM) presented fluctuating values but at the time of abortion values were normal. One patient (VR) showed increasing values from below 10 within the reference area predicting a favourable outcome. At the first blood sam-

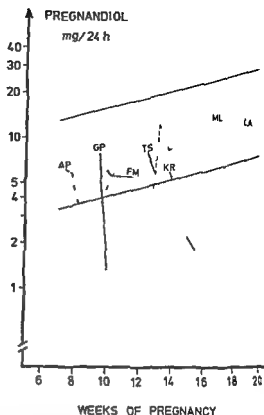


Fig 2 Pregnanadiol in urine in threatened abortion  
 — Pregnancies proceeding to delivery  
 - - - Pregnancies ending in abortion  
 Single values from pregnancies ending in abortion. The 95 per cent reference range is indicated

ple all patients who continued pregnancy values. After serial determinations one patient presented decreased values and later one low predicting unfavorable outcome. The following values increased and were later normal again.

In 50 patients the pregnanadiol excretion in 24 urine specimens were determined. Results are entered on Fig 2. At the first determination five of 15 patients who later aborted showed normal values predicting favorable outcome. Ten patients had low values. After analysis of serial specimens one patient (GP) had low values after an abrupt decrease. In three patients (FM, TS, KR) who showed low levels of pregnanadiol the values were still normal at the time of abortion. Of the 35 patients continuing pregnancy only one (LA) had a low value in the 24 urine specimen. After serial determinations the values were normal. On the other hand another patient showed a sharp decline to a value far below the reference area predicting unfavorable outcome. Following values however were normal.

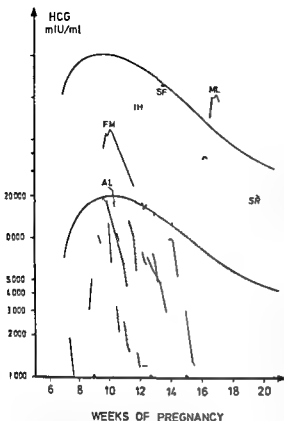


Fig 3 HCG in serum in threatened abortion  
 A Pregnancies proceeding to delivery  
 B Pregnancies ending in abortion  
 Single values from pregnancies ending in abortion  
 The 95 per cent reference range is indicated

HCG in serum was determined in 63 patients the results are shown on Fig 3. None of the 35 pregnancies proceeding until delivery had low values at the first sample. After serial determinations one patient (SR) showed values falling to levels below the reference range. At this gestational age falling levels of HCG are expected but less than found in this patient. The values thus initially predicted unsuccessful outcome but the following values increased and were within the normal range. Concerning the 28 patients who aborted 23 showed low values at the first sample. After serial determinations one further patient (AL) showed low values. Another patient (FM) who eventually aborted presented decreasing levels of HCG but at this time of pregnancy decline in the values was expected.

In Tables I, II and III the predictive values for each hormone are calculated based on the first blood sample and first urine specimen and on serial determina-

tions. Increasing values and/or a rise of concentration into the reference range was regarded as a positive sign predicting favorable outcome of the pregnancy. The contrary a negative sign predicting an unsuccessful outcome.

## DISCUSSION

In previous studies progesterone determinations were found of diverging value in threatened abortion. Kunz & Keller (7) found the test of good prognostic value whereas Nygren *et al* (10) found that progesterone rendered an uncertain prediction mainly because of a great deal of overlapping between the two groups of patients.

In the present study the progesterone values in the patients who subsequently gave birth showed great individual variations from day to day during the first days of admission and from week to week later in pregnancy (e.g. LJ Fig 1). Moreover the spread was large. These findings are in agreement with Lindberg *et al* (9) who investigated 32 healthy pregnant women serially throughout pregnancy. They also found large but non systematic diurnal variations. When a decrease occurs in the progesterone level the problem arises whether it is a token of inevitable abortion or just a normal phenomenon. This may result in misjudgement of the prognosis and could lead to the evacuation of a normal pregnancy. Tulchinsky & Hobel (14) found that the progesterone levels in ten healthy pregnant women did not rise from the 5th to 10th week of pregnancy. Values in this period were only slightly above those observed in the luteal phase of the menstrual cycle. After the 10th week a significant rise was found. Consequently steady levels of progesterone in early pregnancy are not necessarily pathological.

Table 1 The predictive values for progesterone in serum

	First sample		Serial samples	
	Pat's who aborted	Pat's who gave birth	Pat's who aborted	Pat's who gave birth
Low values	18	0	23	1
Normal values	11	35	6	34

Predictive value of a positive test in first sample  $18/18 \times 100\% = 100\%$   
 in serial samples  $23/24 \times 100\% = 96\%$

Predictive value of a negative test in first sample  $35/46 \times 100\% = 76\%$   
 in serial samples  $34/40 \times 100\% = 85\%$

Table II The predictive values for pregnandiol in urine

	First sample		Serial samples	
	Pat s who aborted	Pat s who gave birth	Pat s who aborted	Pat s who gave birth
Low values	10	1	11	1
Normal values	5	34	4	34
Predictive value of a positive test in first sample $10/11 \times 100\% = 91\%$ in serial samples $11/12 \times 100\% = 92\%$				
Predictive value of a negative test in first sample $34/39 \times 100\% = 87\%$ in serial samples $34/38 \times 100\% = 89\%$				

At the first progesterone determination in this study all patients whose pregnancies were successful had values within the reference range i.e. no false positive values were found. At first glance the predictive values for progesterone (Table I) especially that for a positive test seem satisfactory. However 11 of the 29 patients with unsuccessful outcome of pregnancy had values within the reference range at the first blood sample. In other words a considerable overlap was found between the two groups. Because of this we find both single and serial determinations of progesterone of limited value in the evaluation of the prognosis in threatened abortion. On the other hand the results also show that an initial progesterone value below the reference range in every case is followed by spontaneous abortion.

Acevedo *et al* (1) found pregnandiol of prognostic value in threatened abortion. Brown *et al* (2) also concluded that the analysis was of value. They found that the 50th percentile did not exceed the upper limit of the pregnandiol levels of the luteal phase of the menstrual cycle before the 13th week of pregnancy and the 10th percentile not before the 18th week. The pregnandiol values in the present study show the same characteristics in the successful pregnancies as did the progesterone values. Great individual variations from day to day and week to week (e.g. AP Fig 2) a large spread and in early pregnancy normal values not higher than values normally found in the luteal phase of menstrual cycle. There was overlap between the successful and unsuccessful pregnancies. A 24-hour urine specimen demands careful collection. The determination is delayed by the 24 hours occupied by the collection and pregnandiol only reflects a fraction of the progesterone produced. For these reasons we cannot recommend pregnandiol determinations for monitoring threatened abortion. With an

assay for progesterone available we find the tion of pregnandiol obsolete.

Nygren *et al* (10) found that a single determination of HCG in serum gave a very good assessment of prognosis in threatened abortion during the first trimester. Kunz & Keller (7) also found the analysis of value but recommended its use in association with other hormonal parameters. Using a specific receptor assay for HCG Rosal *et al* (12) were able to predict spontaneous abortion as early as eight to ten days after conception.

In early pregnancy there is a very rapid increase in the HCG values reaching peak levels between 10 to 12th weeks. After this an abrupt fall is seen to minimum values between the 20th to 24th weeks. Individual variation is high and the range is large. Decreasing values are normal after the 10th week of gestation. The gestational age must be known accurately if changing HCG values are to be correctly interpreted. Otherwise decreasing HCG values might suggest that the pregnancy is capable of and result in the evacuation of an otherwise viable pregnancy (e.g. SR Fig 3). We consider serial HCG determinations unsuitable for monitoring threatened abortion in spite of its potentially good value (Table III).

All patients continuing pregnancy until delivery had HCG values within the reference range at the blood sample. The predictive value of the first is very good (Table III). Eighteen patients aborted had values below 10 000 mIU/ml after first sample. None of the patients with successful pregnancies showed such low values at any time between 8th and 15th week of pregnancy. Thus it is reasonable to conclude that HCG values lower than 10 000 mIU/ml between the 8th and 15th week of gestation predict spontaneous abortion.

Table III The predictive values for HCG in serum

	First sample		Serial samples	
	Pat s who aborted	Pat s who gave birth	Pat s who aborted	Pat s who gave birth
Low values	23	0	24	1
Normal values	5	35	4	34

Predictive value of a positive test in first sample  $23/23 \times 100\% = 100\%$  in serial samples  $24/25 \times 100\% = 96\%$   
 Predictive value of a negative test in first sample  $35/40 \times 100\% = 88\%$  in serial samples  $34/38 \times 100\% = 89\%$

In the healthy pregnant woman HCG is only produced in the trophoblast in the placenta. From the study of Scommegna *et al* (13) it is likely that the exclusive precursor of progesterone synthesis is maternal cholesterol. The site of the progesterone synthesis either the corpus luteum or the placenta about 15-20 per cent is excreted in the urine as pregnandiol. All three hormones reflect only placental or maternal placental function and tell nothing of fetal function. It is possible with fetal death but with a still functioning placenta to find normal values of all three hormones. Hormones used for monitoring early complicated pregnancy should be of fetoplacental origin.

# ACKNOWLEDGEMENT

I wish to thank bio-statistician cand polit B Olesen and Statens Serum Institut Copenhagen for valuable statistical help.

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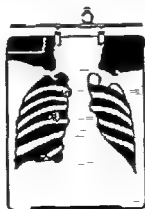
Submitted for publication February 1 1978

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# **DEPO-PROVERA PROVERA TABLETS**

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# THE DIAGNOSTIC VALUE OF ULTRASONOGRAPHY IN 342 SUSPECTED CASES OF ECTOPIC PREGNANCY

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**Abstract** The value of ultrasonography as a diagnostic or screening tool is studied in 342 cases of potential ectopic pregnancy. Ultrasonography was used as a diagnostic technique when the echopattern was clear enough to pose a diagnosis of ectopic or non-ectopic pregnancy including intra uterine pregnancy, abortion or other pelvic abnormalities. Diagnosis was possible in 77 per cent of the examined patients with 95 per cent correct diagnosis, 2 per cent false positives and 3 per cent false negatives. For ultrasonography simply as a screening technique whatever the echopattern might be, in all cases 78 per cent were detected correctly with 20 per cent false positives and 2 per cent false negatives.

The high quality of the ultrasonic data combined with the relative absence of the disadvantages of roentgen rays and invasive techniques makes ultrasonography the first examination of choice in cases of clinical suspicion of ectopic pregnancy.

Except in cases of Fallopian tube rupture with accompanying internal hemorrhage, the diagnosis of ectopic pregnancy (EP) is difficult. When the signs and symptoms of early pregnancy which are not always definite are combined with slight vaginal bleeding, moderate pelvic pain and palpation of a small paracervical mass, EP must be considered among the possible diagnoses. These symptoms which are not necessarily caused by an EP are quite common. They are

often seen in cases of abortion associated with the development of a corpus luteal cyst. When the symptoms are very characteristic of an EP, recourse to complementary examinations is unnecessary. The clinical diagnosis is immediately followed by surgical treatment.

However, the majority of cases are those in which signs of EP are least evident and the diagnosis of EP is just one of many. Here complementary examinations are necessary. Among these, laparoscopy is considered the most useful because it helps avoid unnecessary emergency surgery. No surgical exploration is completely devoid of risks and laparoscopy is no exception. And even if the risk involved is minimal, any laparoscopy requires some hospitalization. Also, laparoscopy cannot be used in every instance because of its inherent contraindications (e.g. previous abdominal or pelvic surgery).

A technique which could be used instead of laparoscopy or which could at least select indications for the use of laparoscopy would be greatly appreciated for its help in resolving the problem posed when EP figures in the differential diagnosis. This technique must meet certain criteria. It must be harmless to the mother and embryo; it must be practicable at any mo-

Table I Annual percentages of ultrasonography indicated for suspected ectopic pregnancy per number of patients examined and per number of examinations respectively

Year	Total no of patients	No of suspected EP	per cent	Total no of examinations	No of suspected EP	per cent
6-68	445	0	0	810	0	0
9	618	5	0.81	1 377	5	0.36
10	1 170	12	1.03	2 400	12	0.50
11	1 466	17	1.16	2 918	19	0.65
12	1 973	32	1.62	3 763	35	0.93
13	2 325	65	2.80	4 500	71	1.57
14	2 600	101	3.88	4 824	113	2.34
15	3 018	69	2.29	5 209	79	1.52
16	3 268	70	2.14	5 228	79	1.51
Total	18 883	371	2.19	31 029	412	1.33

Only A-mode scanner available

Table II Annual results with ultrasonic diagnosis (UD) controlled with invasive techniques (IT), clinical follow up (CF)

Year	No of suspected EP	I EP			II EP Possible			III EP Excluded			IT Diagnosis	
		UD correct (IT +)	UD false+ (IT -)	UD false- (CF)	UD correct (IT +)	UD false+ (IT -)	UD false- (CF)	UD correct (IT -)	UD false+ (CF)	UD false- (IT +)	(EP+)	(EP-)
1969	5	1	-	-	-	-	-	2	1	1	2	2
1970	9	-	-	-	1	1	1	1	2	3	4	2
1971	14	1	-	-	1	3	1	5	2	1	3	8
1972	30	1	2	-	4	10	-	2	11	-	5	14
1973	51	3	1	-	1	8	3	4	38	-	4	13
1974	93	7	2	-	-	8	5	11	59	1	8	21
1975	67	1	1	-	6	9	1	5	39	-	7	11
1976	66	6	-	-	2	3	4	13	36	2	10	16
Total	342	20	5	-	15	42	20	43	188	8	43	91
Per cent	100	5.9	1.8	-	4.4	18.1	-	67.5	2.3	-	12.6	26.6

ment it should not necessitate anesthesia hospitalization or cause undue discomfort to the patient its diagnostic value must be demonstrated. The data obtained by ultrasonography are being appreciated more and more in all medical areas as echographic techniques are improved (e.g. grey tones) and the practitioners become increasingly skilled at interpreting the various abnormalities which can be encountered. To us ultrasound seems to fill all the above criteria leaving only the final criteria of its value in diagnosing EP to be established. Since ultrasonography was proposed for exploring the pelvis (16) attempts to apply this technique to EP diagnosis have given mixed results. These will be discussed later in this study.

We hope that our experience involving 371 cases using ultrasonography when an EP was clinically assumed will show how these techniques might help to solve a difficult diagnostic problem.

## MATERIAL AND METHODS

**Patients** From 1966 till 1976 883 patients referred by the Ultrasound Diagnostic Department, Obstetrics and Gynecology Clinic, University of Brugmann necessitating 31 029 ultrasonographic examinations. Possible EP was the indication for 371 patients (Table I). Twenty nine patients involved in a follow up were withdrawn from the study; the 342 remaining cases form the basis of this study.

It should be noted that ultrasound examinations ruled out 17 other patients for diverse reasons prior suspicion of EP. However a diagnosis of EP was made in the ultrasound exam or final protocol. These are discussed separately.

The validity of ultrasonic diagnoses was assessed by invasive techniques (IT) i.e. laparoscopy and/or in 134 of the 342 cases (39 per cent) or by careful follow up (CF) in the remaining 61 per cent. Eight cases of EP admitted to the clinic and operated on prior ultrasound examination have been omitted from this study.

Table III Ultrasonography as a diagnostic tool (UD) in cases with clear echopattern (i.e. in 265 out of 342 cases or 77 per cent)

Year	No of suspected EP	No with clear echo	UD correct		UD false+		UD false-	
			No	Per cent	No	Per cent	No	Per cent
1969-72	51	36	29	81	2	6	5	14
1973	51	46	45	98	1	2	0	0
1974	93	80	77	96	2	3	1	2
1975	67	46	45	98	1	2	0	0
1976	66	57	55	96	0	0	2	4
Total	342	265	251	95	6	2	8	3

Table IV Ultrasonography as a selective tool (i.e. for the selection of EP in 100 per cent of cases)

Year	No of suspected EP	EP+						EP-						Total					
		Total	No	%	Correct	No	%	Total	No	%	Correct	No	%	False-	No	%	Correct	F+	F-
69-72	55	27	46.6	9	33.3	18	66.7	31	53.4	26	83.9	5	16.1	35	60.3	31.0	8.6		
1973	58	16	27.6	4	25.0	12	75.0	42	72.4	42	100.0	0	0	46	79.3	20.7	0.2		
1974	93	22	23.7	7	31.8	15	68.2	71	76.3	70	98.6	1	1.4	77	82.8	16.1	1.1		
1975	67	23	34.3	7	30.4	16	69.6	44	65.7	44	100.0	0	0	51	76.1	23.9	0.0		
1976	66	15	22.7	8	53.0	7	47.0	51	77.3	49	96.1	2	3.9	57	86.4	10.6	3.0		
Total	34*	103	30.1	35	34.0	68	66.0	239	69.9	231	96.7	8	3.3	266	77.8	19.9	2.3		

## RESULTS

**Techniques** The principles and techniques of ultrasonic examination as discussed in numerous references are now well known (3, 7, 15). Examinations were carried out with the following equipment: ALOKA (SSD 60 BISTABLE SYSTEM 2.25 MHz) and KRETZ TECHNIK (Combison BISTABLE SYSTEM THEN Combison II GREY SCALE 2 MHz). The scans were recorded on Polaroid film or on 4010 Tektronix hard copier. Some figures presented here were obtained in 1977 with the SEARLE 2050 sonic (Grey Scale) equipment thus after the above mentioned period.

**Echographic criteria** The most characteristic echographic pattern of EP is as follows: The uterus is slightly enlarged or normal. The thickened endometrium or some blood in the uterine cavity reflects strong echoes. Close by the uterus a mass can be seen which generates ring shaped echoes. This represents the gestational sac. Embryo can be displayed provided the pregnancy has progressed at least to the stage of 7-8 weeks/LMP (Fig 1-4).

Sometimes a mass is visible behind the uterus in the posterior fornix. When the mass is regularly outlined and anechoic it corresponds to accumulation of blood in the Douglas pouch when irregular and filled with echoes to a thickened tube and blood clot (Fig 5-6).

The above mentioned echogram is protocolled as **EP highly probable**. If despite all indications the diagnosis cannot be made with absolute certainty it is because we feel that only macro- or microscopic examination will verify the presence of an EP.

Because this typical echo-pattern is rarely encountered following less typical images must be considered.

**EP probable** when the echogram is composed of a faint uterine mass with some echoes vaguely arranged in a ring echo-pattern next to a well-defined non gravid uterine tube (Fig 7, 10).

**EP possible** is used for patients with evidence of early pregnancy when a mass is found next to the uterus but without any ring echo-pattern inside (Fig 11, 13).

**EP excluded** a diagnosis of EP can be reasonably excluded if the gestational sac is visible in the uterus or if the echogram is normal even in the face of symptoms of early pregnancy (Fig 14, 15). Other diagnoses are given in some cases for example evolving pregnancy, missed abortion, complete or incomplete abortion, ovarian cyst etc (8).

To simplify the presentation of the data we have divided the cases into 3 major groups formed from conclusions gathered via ultrasonic examinations (Table II).

**Group I or EP** all cases labelled **EP highly probable** and **probable**.

**Group II or EP possible** cases where EP was mentioned only as a simple possibility accompanied by either a recommendation of careful surveillance or laparoscopic examination.

**Group III or EP excluded** cases where echography showed EP to be highly improbable.

These groups were subsequently subdivided as a function of the final diagnosis (correct false positive or negative) and further information is given regarding the method used to verify the ultrasonic diagnosis: 1) Invasive techniques (IT) subdivided into EP positive and EP negative depending on the results obtained. 2) clinical follow up (CF) (spontaneous abortion, absence of pregnancy or evolutive pregnancy).

We find that of 26 ultrasonic diagnoses of EP 20 were exact and 6 false positives. In 239 ultrasonic diagnoses of EP excluded 231 were exact and 8 false negatives. Among 77 cases diagnosed EP possible by ultrasound 15 were actually found to be EPs.

In 17 out of the 8 646 patients examined ultrasonically during the last 3 years of the above mentioned period for reasons other than suspicion of EP a diagnosis of EP was posed either by ultrasound or later by other means. In these cases an ultrasonic diagnosis of EP was made in 14 4 correct. 3 were false positive and the remaining 3 were false negative.

Ultrasonography's utility in cases suspected of being EP can be considered in the following lights:





Fig 1 6 weeks gestational sac located in ectopic position visible in the Douglas pouch (Combison II)  
B bladder C balloon of Foley catheter  
S gestational sac U uterus

**A Ultrasonography as a diagnostic procedure** (Table II) A sufficiently precise echo pattern enables an unequivocal diagnosis. This entails a greatly reduced risk of error. Ultrasonography may be regarded as a means of diagnosis since a diagnosis considered as certain was made in 285 out of 342 cases (77.5 per cent) (groups I and III). The diagnosis was correct in 151 out of these 265 cases (94.7 per cent) while 6 were



Fig 2 12 weeks gestational sac and fetus above the uterus (Combison I)  
E embryo S gestational sac U uterus



Fig 3 Ectopic pregnancy 9 week-old embryo (Combison II)  
E embryo S gestational sac

false positives (2.3 per cent) and 8 were false negatives (3.0 per cent).

Echo patterns showed pelvic anomalies but sufficiently characteristic of EP (group II) in 342 cases (22.5 per cent). These anomalies found to be an EP in 15 of the 77 cases (19.5 per cent).

**B Ultrasonography as a method of selecting pregnancy risk** (Table IV) Bearing in mind cases referred for ultrasonography were selected on the basis of the history and phys-



Fig 4 Embryo's body movement displayed by the same case as Fig 3 (Combison II)  
A maternal abdominal wall E embryo's echo  
S gestational sac diameter



Fig 5 Ectopic pregnancy enlarged right tube containing pattern echoes fluid filled Douglas pouch (Combison I)  
B bladder F fluid G gestational sac T tube U uterus

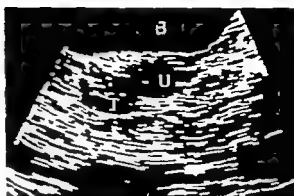


Fig 7 Ectopic pregnancy 5 weeks Enlarged right tube containing irregular ring pattern echoes (SSD 60)  
B bladder U uterus T tube

mination one would expect subsequent ultrasonic examination to confirm or deny the risk of EP. In the former instance follow up would entail laparoscopy or laparotomy. In the latter the patient would be reassured and sent home. Seen in this way ultrasounds confirmed the clinical risk of EP in only 103 of the 12 patients examined or 30.1 per cent (Groups I and II) indeed 31 cases were EP i.e. 34 per cent of the ultrasound risk group. When ultrasonography

allowed us to estimate as unlikely or nil the risk of EP in 239 of 342 cases or 69.9 per cent (Group III) the risk was incorrectly eliminated in 8 patients i.e. 3.35 per cent of the ultrasound free-of-risk group.

Whether ultrasounds were used for diagnosis or solely for screening suspected EPs the number of false positives greatly exceeded the number of false negatives (19.9 per cent versus 3 per cent). Here the cases from the group *EP possible* were placed with those classed as certain diagnoses. The discrepancies between false positives and negatives are explained by the prudence exercised by the ultrasonographer the



Fig 8 Ectopic pregnancy 6 weeks Enlarged left tube and gestational ring (arrow) (Combison I Hard Copy)

6 Ectopic pregnancy 4 selected scans showing enlarged left tube 5 weeks gestational sac uterine cavity (10-Sonic)  
A transverse and B C longitudinal scans  
G gestational sac U uterus  
T right tube



Fig 9 Ectopic pregnancy 7 weeks. Gestational sac and embryo located in the interstitial portion of the right tube (Combison I)

E embryo S gestational sac U uterus

consequences of a diagnostic error leading to lack of treatment can be disastrous.

The chronologic presentation of data beginning in 1969 is designed to evaluate the evolution of these results (Table III and IV). Data from the years 1969–72 have been grouped together in view of the minimal number of ultrasonic examinations that were performed for EP in that period. One sees that the rate of correct results steadily increases with a corresponding decrease in false positives and negatives among the doubtful cases. This evolution can be explained by the experience gained with ultrasonography while the inconsistency in the year to year



Fig 10 Ectopic pregnancy 5 weeks. Compare the echoic pattern of the uterus (U) and the anechoic pattern of the gravid left tube (Combison II)



Fig 11 Non typical echo-pattern of ectopic pregnancy. Irregular outlined mass right to the uterus (U) (Combison Hard Copy)

improvement is due to the rapid turn-over of staff in the Department.

## DISCUSSION AND CONCLUSION

Many papers deal with ultrasound and EP, only 11 present results (2, 3, 4, 9, 11, 12, 13, 14, 16) and 11 have been included in Table V.

Most of the others (in 9, 10, 14, 16) report case by case. When studies are based on a small number of cases, the level of correct diagnoses can conceivably exceed 90 per cent. It seems to us that selective cases is radically different and for this reason we feel that comparison of data would not be useful. They are given only as examples.



Fig 12 Non typical echo-pattern of left ectopic pregnancy (Pho-Sonic). A longitudinal scan B transverse scan U uterus

Table V Results literature data on results with ultrasonic diagnosis (UD) for suspected EP

Year	Authors	No of suspected EP	UD Correct				Total %
			EP No	EP %	Others No	Total No	
1969	Kobayashi <i>et al</i> (4)	39	16	41	13	29	74
1970	Zacutti <i>et al</i> (16)	30	22	73	6	28	93
1971	Jouppila (3)	17	3	18	8	11	65
1972	Suk <i>et al</i> (12)	10	7	70	—	7	70
1972	Varma (13)	20	5	25	13	18	90
1973	Schlensker (11)	47	10	21	34	44	94
1976	Walch <i>et al</i> (14)	52	11	21	33	44	85
1976	Levi (9)	87	14	16	46	60	69
1976-77	Hassid (2)	65	13	20	46	59	91
Present study		342	35	10	231	266	78

Table V continuation

JD incorrect			Verified EP		
size+	No	False— No	Total %	No	Per cent
5	5		26	21	54
2	—		7	22	73
—	6		35	9	70
3	—		10	7	70
1	1		10	6	30
1	2		6	12	26
6	2		15	13	25
4	3		32	17	20
2	4		9	17	26
8	8		22	43	13

We are convinced that an improvement in the results can be achieved by reducing the number of false positives and negatives. We believe this is possible by repeating ultrasonic examinations more often when possible and by requiring the ultrasonographer to perform a systematic vaginal examination.

3 by making sure that the ultrasonic examination is performed under optimum conditions i.e. bladder properly filled, use of high-quality ultrasonic equipment manned by an experienced ultrasonographer.

It must also be mentioned that the diagnostic value of invasive techniques which are not repeatable in the event of failure depends on similar factors i.e. anatomical condition, the quality of materials and the capacity of the examiner.



Fig 14 Intra uterine pregnancy 5 weeks (Pho-Sonic). Arrows shows the gestational sac.

13 Non typical echo-pattern of left ectopic pregnancy and tube lies in the Douglas pouch (arrow) (Pho-Sonic).



Fig 15 Normal pelvis 6 weeks amenorrhea slight bleeding and right mass palpated (O=right ovary U=uterus) (Pho-Sonic)

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Submitted for publication May 2 1978

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# URINARY ESTROGEN EXCRETION AND CONCENTRATION OF SERUM HUMAN PLACENTAL LACTOGEN IN PREGNANCIES FOLLOWING LEGALLY INDUCED ABORTION

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**Abstract** Feto-placental function was assessed by 24-hour excretion of estrogens in urine and by the concentration of human Placental Lactogen (hPL) in serum in pregnant women whose previous pregnancy was terminated by legally induced abortion. The mean 24-hour excretion of estrogens in urine and the mean concentration of hPL in serum were lower in this group than in women without previous induced abortion. Neither was the frequency of a low 24-hour excretion of estrogens in urine or low concentration of hPL in serum (values less than mean  $\pm 1.96$  s) found to be increased.

This study could not demonstrate an increased frequency of dysfunction of the feto-placental unit during the last part of pregnancy in women with previous legally induced abortion. These findings indicate that legal abortion does not seem to increase the frequency of retarded intrauterine growth in a subsequent pregnancy.

Abortion generally includes instrumental dilatation of cervix uteri followed by an evacuation of the uterine cavity either by the suction method or by curettage. Both procedures may cause lesions of the uterus either as lesions of the cervix or lesions leading to defects in the uterine cavity or synechia as demonstrated by Krayl and Lavric (13) and von Seewald *et al.* (18). It remains to be clarified if such lesions increase the risk of prematurity in a subsequent pregnancy either because of cervical insufficiency or because of compromised placental function leading to retarded intrauterine growth. Harlap and Davies (10) found an increased risk of prematurity in women who previously had an induced abortion. However, this was not confirmed by Dalung and Emanuel (6) (16) nor Roth and Aoyama (19).

This study tries to clarify whether feto-placental function is seen more frequently in women whose previous pregnancy had been terminated by a legally induced abortion by assessing urinary excretion of estrogens and the concentration of serum human placental lactogen (hPL) during the last part of pregnancy.

## MATERIAL AND METHODS

The study included 576 women whose previous pregnancy had been terminated by a legally induced abortion registered during the period 1st April 1974 to 31st December 1975 for delivery at the Rigshospitalet and Frederiksberg Hospital in Copenhagen. These women, group 1, were compared with different control groups selected from among the other women registered for delivery during the same period.

The following control groups were described:  
**Group 2** All women whose previous pregnancy had ended in a spontaneous abortion or stillbirth (1 009).

**Group 3** Women whose previous pregnancy had ended in delivery of a live child (539).

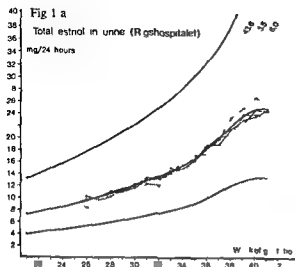
**Group 4** Women with no previous pregnancies (337).

Groups 3 and 4 were matched to group 1 using the matched pair technique. Age (5 year groups), socio-economic status and parity were used as matching criteria. Women in group 1 with only one previous pregnancy terminated by a legal abortion were matched with women of group 3 with parity 1 in addition to women in group 4. As group 3 is smaller than group 1 suitable matches for all women in group 1 were not obtainable from group 3.

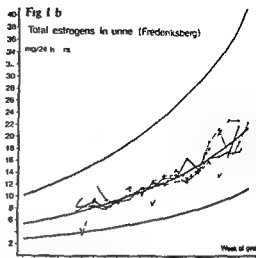
An analysis of the population as well as the matching procedure adopted have previously been described (Obel (16, 17)).

We aimed at measuring the excretion of estriol in urine and the concentration of hPL in serum at least twice during the pregnancy. It was not possible to measure the hormones mentioned twice in all patients as some moved districts and some did not want to take part in the study. The study also included all hormonal values undertaken for an obstetrical reason. The matching procedure was rather protracted and not until afterwards did we learn which women were included in groups 3 and 4. Owing to this delay the number of measurements in group 3 and 4 is smaller than in groups 1 and 2.

The two hospitals adopted different methods for measuring the excretion of estriol in urine. At Rigshospitalet the 24-hour excretion of estriol in urine was tested according to the method described by Frandsen (7). At Frederiksberg Hospital they adopted the method described by Brown (5) to measure the total amount of estrogen in urine. (In the last part of pregnancy estriol amounts to 95 per cent of the total estrogens measured (Adlercreutz and Luukainen (1)).



**Fig 1 a b** Excretion of estriol (in Fredenksberg Hospital total estrogen excretion) in urine during the last part of pregnancy in women grouped according to the outcome of the previous pregnancy. The figure shows the mean values in the following groups:  
— Group 1: Women whose last pregnancy was terminated



by legally induced abortion  
— Group 2: Women whose last pregnancy ended in neonatal abortion or stillbirth  
Group 3: Women whose last pregnancy ended in a live child  
Group 4: Women with no previous pregnancies

## RESULTS

563 women in group 1 delivered and 13 spontaneously. In group 2 967 delivered 523 3 and 328 in group 4, whereas there were 42 spontaneous abortions respectively.

In group 1 91.9 per cent in group 2 90.8 per cent in group 3 and in group 4 82.8 per cent had urinary estrogen excretion measured at least once during the pregnancy. Serum hPL was estimated once for 89.9 per cent in group 1 89.0 per cent in group 2 82.6 per cent in group 3 and in group 4 80.9 per cent.

When comparing the number of women in the groups with 1, 2, 3 or more hormone tests we found that more measurements had been made in group 1 and 2 than in groups 3 or 4,  $p < 0.001$ .

Fig 1 indicates the mean 24-hour urinary estriol in urine (at Fredenksberg Hospital total estrogen) in each of the groups 1-4. The reference area is also marked in the figure. Fig 2 has equivalent rates as regards hPL. As it appears from these figures we did not find any difference in values of either excretion of estrogen nor of hPL between group 1 and the other groups.

Table 1 includes women in groups 1, 2 and 3 with only one previous pregnancy as well as women in group 4. It shows the number of women with at least one hormone value below the lower limit of the reference area, as well as the number of women

The concentration of hPL in serum was also measured by different methods in the two hospitals. Rigshospitalet adopted the method described by Gæde and Nørgård-Pedersen (9), whereas Fredenksberg Hospital used the method of Lebech and Borggård (14).

Because of the different methods used at the two hospitals the results from each hospital have been analysed separately.

For statistical analysis we presumed that the excretion of estriol in urine as well as the concentration of hPL in serum had a logarithmic normal distribution in each week. This assumption was confirmed by histograms of the values measured for each gestational week as well as fractile diagrams of the logarithm of the observations.

Mean hormonal values in women delivering only one child weighing more than 2500 grams were in each gestational week compared with equivalent values in women delivering only one child over 2500 grams without any pregnancy complications. This comparison revealed no systematic differences. In order to define the reference area with as great an accuracy as possible the calculations were then based on the considerably larger group of women who had delivered only one child weighing more than 2500 grams.

The reference area was determined by calculating the mean and the variance of the logarithm of the observations for each pregnancy week from week 21-41. The curve obtained has a linear trend from week 21 to 33. A smoothed curve of mean values was then determined by means of a combination of linear regression up to week 33 and then in the last part of pregnancy by moving average. The reference area is in each pregnancy week based upon 95 per cent confidence intervals.

Human Placental Lactogen (Rigshospitalet)

Fig 2 a

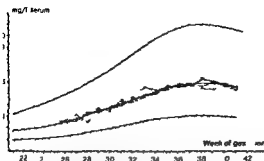


Fig 2 a & b Mean concentration of serum human Placental lactogen during last part of pregnancy in women grouped according to the outcome of the previous pregnancy  
 - Group 1 Women whose last pregnancy was terminated legally induced abortion

1 values above this limit during the intervals 30-33 1-37 and 38-41 weeks gestation

The number of women in group 1 with at least one hormone level below the lower limit of the reference area during the 4-week intervals mentioned was not significantly greater than in groups 2, 3 and 4 ( $p < 0.025$  (Fisher's exact test)). Within each of the week intervals we then determined whether the number of hormone tests differed between the groups. More measurements of urinary excretion of estrogen as well as of serum hPL had been carried out in groups 1 and 2 than in groups 3 or 4, but the difference was not significant ( $\chi^2$   $p > 0.05$ ).

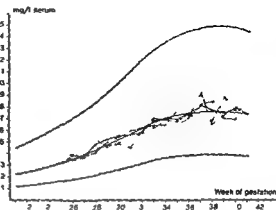
Table II shows the number of women with at least one hormonal value below the lower limit of the reference area (2.5 percentile) in women whose infants weighed under and over 2500 grams respectively. In all 4-week intervals we found a significantly higher incidence of women with low values among those delivering infants with a birth weight below 2500 grams than among the women delivering infants with a higher birth weight ( $\chi^2$   $p < 0.05$ ).

## DISCUSSION

The purpose of the present study was to determine if retarded fetal growth could be demonstrated in women whose previous pregnancy was terminated by a legally induced abortion. Koller and Eikhom (12) found evidence of fetal growth limitation in women with a previous legally induced abortion. In this study they found that infants born after 36 weeks of gestation had a significantly lower birth weight than infants of other women; the difference was especially

Human Placental Lactogen (Frederiksberg)

Fig 2 b



- Group 2 Women whose last pregnancy ended in spontaneous abortion or stillbirth

Group 3 Women whose last pregnancy ended in delivery of a live child

Group 4 Women with no previous pregnancies

marked after 41 weeks of gestation and the authors took this as a sign of growth limitation in women with a previous induced abortion.

Excretion of estrogen in urine (Beicher and Brown (3), Bjoro (4) and Frandsen *et al* (8)) as well as the concentration of hPL in serum (Bellman and Lang (2), Hartog (11) and Lindberg and Nilsson (15)) have previously been described as being correlated with fetal growth values often being low in cases of retarded fetal growth. This study also showed that excretion of estrogen in urine as well as the concentration of hPL in serum correlated with birth weight so that low values were more frequently demonstrated in women delivering infants with birth weight below 2500 grams than in women delivering infants with birth weight over 2500 grams.

The analysis of the mean excretion of estrone in urine (at Frederiksberg Hospital total estrogen) and the mean concentration of hPL in serum did not demonstrate an increased frequency of fetoplacental dysfunction in those women whose previous pregnancy had been terminated by a legal abortion.

Even so it might still be possible for instance if the distribution of the values observed varied between the groups assessed that fetoplacental dysfunction might appear more frequently in pregnancies following legal abortion compared with others.

In order to investigate this problem we have for 4-week cohorts in the last part of pregnancy estimated how many women within each cohort had values below the lower limit of the reference area (2.5 percentile).



Table 1 Women with excretion of estrogen in urine and concentration of human Placental Lactogen in s below the 2.5 percentile during last part of pregnancy. The women are grouped according to the previous pregnancy. Group 1, 2 and 3: Women with only one previous pregnancy terminated by legal induced abortion, spontaneous abortion or stillbirth, and delivery of a live child respectively. Group 4: with no previous pregnancy.

Gestation Week	Group 1 Hormone level			Group 2 Hormone level			Group 3 Hormone level			Group 4 Hormone level		
	All observations	At least one observation†	No. %	All observations	At least one observation†	No. %	All observations*	At least one observation†	No. %	All observations*	At least one observation†	No. %
<b>Estrogen in urine</b>												
Rigshospitalet												
30-33	243	5	3.4	251	11	4.2	101	2	1.9	73	6	7.5
34-37	153	11	6.7	254	17	6.3	168	7	4.0	136	11	8.1
38-41	116	10	7.9	164	9	5.2	136	9	6.2	119	11	8.8
Frederiksberg Hospital												
30-33	46	1	2.1	46			32	2	5.9	20		
34-37	45	1	2.2	47	1	2.1	67	1	1.5	58	3	4.9
38-41	10			15	3	16.7	20	1	4.8	25	3	10.7
<b>hPL in serum</b>												
Rigshospitalet												
30-33	151			248	9	3.5	74	2	2.6	61	2	3.2
34-37	152	3	1.9	245	12	4.7	175	1	0.6	135	3	2.2
38-41	99	3	2.9	131	11	7.7	120	5	4.0	108	2	1.8
Frederiksberg Hospital												
30-33	45			47	1	2.1	57	3	5.0	47	1	2.1
34-37	48	1	2.0	49	1	2.0	68	2	2.9	63	2	2.9
38-41	11	2	15.3	13			31			24	1	4.0

\* > mean - 1.96 s

† ≤ mean - 1.96 s

Groups 1-3 have been described according to the outcome of the previous pregnancy, whereas women in group 4 had no previous pregnancies, therefore parity varies between our groups. It has been demonstrated previously that the number of previous pregnancies was higher in groups 1 and 2 than in group 3, and that the frequency of low birth weight as well as of short gestational age increased with increasing parity, and with the number of previous pregnancies (Obel (17)). Because of the difference mentioned between the groups we decided for analysis of the group of women with low hormonal values only to study those with one previous pregnancy and women in group 4.

The number of women with at least one low value of a hormone measured will, by chance, increase with the number of tests performed. The correlation between the number of tests and the number of women with at least one low value in this study further accentuated, because often a low hormone value, but still within the reference area, may be followed by additional measurements, as the woman in question is

looked upon as an obstetrical risk patient.

During the intervals 30-33, 34-37 and 38-41 gestation, the number of hormone assays in groups 1 and 2 was found to be a little higher, but significantly higher than in groups 3 and 4. The difference in the number of measurements indicates that more women with at least one value below the lower limit would be expected to be in groups 1 or 2 than in groups 3 or 4. But, in spite of this, we did not demonstrate significantly more women in group 1 with at least one hormone assay below the lower limit of the reference area (Fisher's exact test,  $p > 0.05$ ).

## CONCLUSION

The present study could not demonstrate an increased frequency of low urinary excretion of estrogen (Frederiksberg Hospital total estrogen) or low concentration of serum hPL in women whose previous pregnancy was terminated by a legal abortion when compared with control. This finding does not support the theory that retarded fetal growth is more frequent after induced abortion.

Table II The correlation between the excretion in urine as well as the concentration of human Placental Lactogen in serum below the 2.5 percentile during the last part of pregnancy for women with normal and low birth weight infants

	Gestation Weeks	Rigshospitalet Birth weight		Frederiksberg Hospital Birth weight	
		≤2 500 g No. of women with values <mean 1 96 s	>2 500 g No. of women with values <mean 1 96 s	≤2 500 g No. of women with values <mean 1 96 s	>2 500 g No. of women with values <mean 1 96 s
estrogen in urine	30-33	13 13.0%	35 3.3%	1 12.5%	8 3.1%
PL in serum	30-33	8 7.8%	26 2.6%	1 8.3%	6 2.0%
estrogen in urine	34-37	11 22.8%	70 5.5%	3 23.0%	16 5.0%
PL in serum	34-37	16 18.2%	27 2.2%	3 20.0%	7 2.0%
estrogen in urine	38-41	14 32.6%	53 5.4%	3 42.9%	7 5.7%
PL in serum	38-41	6 16.7%	28 3.3%	1 12.5%	4 3.4%

estrogen in urine In Rigshospitalet measured as excretion of estron in urine per 24 hours. In Frederiksberg Hospital measured as the total estrogen excretion in urine per 24 hours

## ACKNOWLEDGEMENTS

The study has received financial support from the Danish Medical Research Council

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Submitted for publication July 27 1978

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# HISTOLOGICAL APPEARANCES OF THE HUMAN PLACENTA OBSERVED BY ELECTRON MICROSCOPY AFTER HYPERTONIC SALINE ABORTION

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**Abstract** Placentae obtained after Hypertonic Saline Induced Abortion (HSIA) were compared with placentae delivered by hysterotomy between 16 and 20 weeks of pregnancy. When investigated by routine light microscopy the outer part of the placental tissue seemed to be normal. Changes were confined to a thin subchorionic layer. Transmission and scanning electron microscopy revealed serious damage especially to the syncytium, not only in the subchorionic zone but also in the transitional and in the decidua. *In vitro* experiments demonstrated that a very short exposure to hypertonic solution can produce impressive histological changes in placental tissue.

abortifacient dose of prostaglandin  $F_{2\alpha}$  decreased the plasma progesterone level and reduced the instillation abortion time.

The third field of controversy concerns the results of microscopic examination of the placenta after hypertonic saline abortion. Several investigators have found lesions of the subchorionic layer of the placenta (2, 4, 10, 13), the most extensive investigations having been made by Christie *et al.* (4). These authors observed characteristic lesions in 14 placentae after intraamniotic injection of hypertonic saline. These lesions were confined to a thin subchorionic layer and even in the most severely affected cases 100 per cent of the placenta was apparently undamaged. They concluded that it is improbable that an intraamniotic injection of hypertonic saline induces abortion by causing extensive damage to the placenta and therefore removing the progesterone block. These conclusions, based on investigations with routine light microscopy up to now, have hardly been challenged. The only published investigation using electron microscopy is to the best of our knowledge a very short and preliminary communication in an article by Wynn (26) and the conclusions are not essentially different from those of Christie: destruction of the fetal surface and maintenance of relative ultrastructural integrity in the maternal zone.

The aim of this study was to investigate placentae obtained by hypertonic saline induced abortion (HSIA) and by hysterotomy at the same gestational age. The investigation, carried out by means of transmission and scanning electron microscopy, was intended to determine the changes in the placenta associated with the instillation of hypertonic saline.

## MATERIAL AND METHODS

The placentae of 17 patients aborted by HSIA were compared with 14 placentae delivered by hysterotomy between 16 and 20 weeks of pregnancy. Immediately after delivery of

the mechanism of initiation of uterine contractions following intrauterine injection of hypertonic saline or other hypertonic solutions has been completely elucidated up to now. In the 1950s Csapo formulated his theory (1, 5, 6) which caused much controversy. According to Csapo, hypertonic solutions could damage the placenta and with its endocrine function. This would then lead to progesterone withdrawal and abolition of the progesterone block.

His theory has been challenged in three different fields of investigation. In the field of endocrinology, one concerning urinary pregnanediol excretion and maternal levels of progesterone and other placental hormones after instillation of hypertonic saline remained controversial (7, 8, 14, 15, 16, 18, 20, 22, 23).

The second field of controversy concerns the prostaglandins. The work of Gustavii (11, 12) suggests that one of the factors involved in the mode of action of intrauterine hypertonic saline is the release of prostaglandins from damaged decidua cells. This never need not be considered as contradictory to the theory of progesterone withdrawal but could be complementary to it. Walker *et al.* (25) observed that the addition of hypertonic urea to an intraamniotic



**Fig 1** Micrograph of a placenta obtained by hysterotomy. Some of the villi are studded with red cells (asterisk) whereas red cells can be observed in other villi (arrows)

This phenomenon is very characteristic of a placenta obtained by hysterotomy

the placenta tissue blocks were cut from the subchorial intermediate and decidual parts. All placentae were studied by light microscopy. In the examination by electron microscopy 5 placentae obtained after hysterotomy and 5 obtained after HSIA were included. From every placenta three blocks were studied and from each block several grids were examined.

The tissue to be examined by light microscopy was placed in Susa fixative and embedded in paraffin. Sections 5 to 6  $\mu$ m in thickness were stained with haematoxylin and eosin, Masson trichrome stain or Alcian Blue PAS Reaction. Tissue samples for Transmission Electron Microscopy (TEM) were fixed in a mixture of 2.4 per cent glutaraldehyde in equal volumes of water and phosphate buffer (0.15 M, pH 7.4) for 3 to 4 hours. Specimens were embedded in Epon 812 using routine methods. Sections were stained with lead citrate and uranyl acetate and observed in a Philips EM 300 at 80 KV. Fixation of the specimens for scanning Electron Microscopy (SEM) was as for Transmission EM. Specimens were submitted to critical point drying (CPD) via amylacetate and  $\text{CO}_2$ . They were then coated with gold in an electron gun evaporator and observed in a Coates and Welter Cwiscan Nr 100 at 10 KV.

One placenta obtained by hysterotomy received special treatment consisting of immersions in different salt concentrations to determine the immediate effects of hypertonic

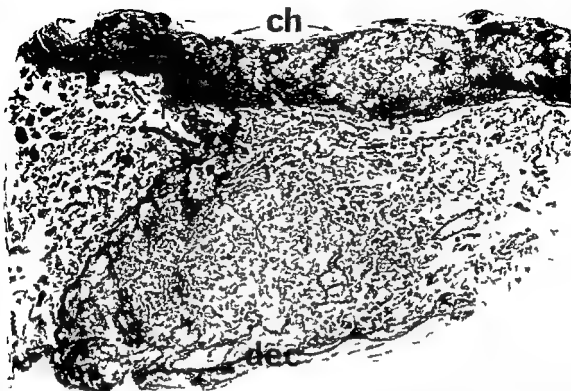
salt solution. Tissue blocks from this placenta as follows:

- a fixed immediately as described above
- b placed for 1/2 hour in Seligman's balanced salt (SBSS)
- c placed for 1/2 hour in a 2 1/2 per cent solution of SBSS
- d placed for 1/2 hour in a 5 per cent solution of SBSS

Subsequently the specimens b to d were fixed for transmission and scanning electron microscopy. An experiment was carried out on 5 blocks of placenta, which were studied afterwards by TEM and SEM.

## RESULTS

Within their groups (hysterotomy, HSIA, etc.) the placentae showed a very uniform picture. **Light microscopy** In the placentae obtained by hysterotomy the vessels in the placental villi showed strong overfilling with blood. Sometimes the vessels were ruptured so that the villi were filled with erythrocytes (Fig 1).



2 Transverse section of a placenta obtained after hypertonic saline abortion. In the upper region a small part of amnio-chorion (ch) is visible. Just below the chorion

is plate (ch) there is a necrotic zone (---). As is shown the thickness of this necrotic zone is variable. In the lower part the decidua basalis (dec) can be observed. HE  $\times$  20.

cross sections of placentae obtained by HSIA a irregular zone of red thrombus is seen directly with the chorion (Fig 2). This thrombotic zone may run up to 20 per cent of the section. Histological abnormalities were confined to the thrombotic zone of placenta whereas the intermediate and decidua basalis had a normal appearance. In the damaged zone the nuclei were pyknotic and there was a variable degree of vacuolization of the cytoplasm. We also observed infiltration by many polymorphonuclear leukocytes as was described by Christie *et al.* (4).

#### Placentae obtained by hysterotomy

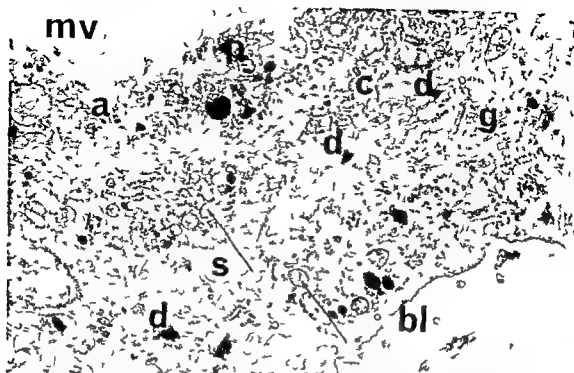
**Transmission EM** At this stage of gestation the cytotrophoblast still consists of two layers which can be clearly distinguished (Fig 3). The apical zone of the syncytiotrophoblast is called the zone of resorption (5) and contains a great number of microvilli, plasma vacuoles, dense bodies and mitochondria; whereas the region that is occupied mostly by lamellae of the RER is called the zone of secretion.

Below the syncytium the cytotrophoblast or Langhans layer is found resting on a very well developed basal lamina. Between the syncytium and the Langhans layer there is a widening of the intercellular cleft into which microvilli project. The cytotrophoblast cells contain cytoplasm which is less electron-dense than the cytoplasm of the syncytial layer; they contain well developed Golgi complexes, very large mitochondria, polyribosomes and many glycogen particles (Fig 3).

**B Scanning EM** The specimens studied consisted of about 8–10 villi and their ramifications (Fig 4). Higher magnifications revealed a good impression of the long slender appearance of the normal microvillous border (Fig 5). The microvilli were often seen to terminate in a small bulbous tip, a phenomenon we also noticed in transmission electron microscopy.

#### Placentae obtained by HSIA

**a Transmission EM** In these placentae it was important to separate the samples into those obtained



**Fig 3** Section through the trophoblast of a placenta of 16 weeks gestation obtained by hysterotomy. The upper left region shows a part of the syncytium with the microvilli (mv) a protrusion (p) absorption vacuoles (a) mitochondria (arrows) cisternae of the RER (c) with ribosomes on their membranes. Between the syncytium and the cytotrophoblast intercellular spaces (s) can be distinguished.

trophoblast has an electronluculent cytoplasm in developed Golgi complex (g) and many free pol and glycogen particles can be observed layers desmosomes (d) are visible. The c on a well-developed basal lamina (bl)  $\times 15\ 000$

from the subchorial region and from the decidual region because after instillation of hypertonic saline solution a concentration gradient exists from the amniotic sac towards the decidual plate. In the subchorial zone where the lightmicroscope revealed serious damage also by electron microscopy the greatest signs of disintegration were found. The cisternae of the endoplasmic reticulum were greatly enlarged the mitochondria were either swollen or their matrices had become very electron dense microvilli appeared as torn membranes and the nuclei had become pyknotic. The first sign of disintegration of a microvillus is a pronounced bulbous swelling even ballooning of its tips. This stage is rather rare in the subchorial area where it is almost always succeeded by a total disintegration and loss of the microvilli.

By distension of the endoplasmic reticulum the cell is becoming rather disrupted and large portions of the cytoplasm bulge as protuberances from the apical border (Fig 6).

In the decidual zone parts of the sometimes found to appear quite normal inspection showed however that here also parts of the endoplasmic reticulum were more than usual and the mitochondria had electron dense matrix as their cristae. Almost all parts of the trophoblastic layer decidual zone however were much more especially the microvillous border (Fig 7) microvilli having become swollen with loss of structure in addition the RER contained distended cisternae.

#### *b Scanning EM*

**Subchorial zone** In the scanning electron microscope we observed that the slender appearance of the villi had disappeared large swellings protruded from the villous surface (Fig 7) and large areas had a plaque appearance. The microvilli appeared to be together to form so called plaques. In addition to the protuberances the subchorial ballooning effect



Scanning micrograph of a part of the human placenta weeks. At this low magnification the ramification of

the villi can be seen. Between the villi small aggregations of red cells and fibrin are distinguishable (arrow)  $\times 400$



Scanning micrograph of a part of a placental villus microvilli have a slender appearance sometimes they

have a bulbous tip  $\times 65\ 000$



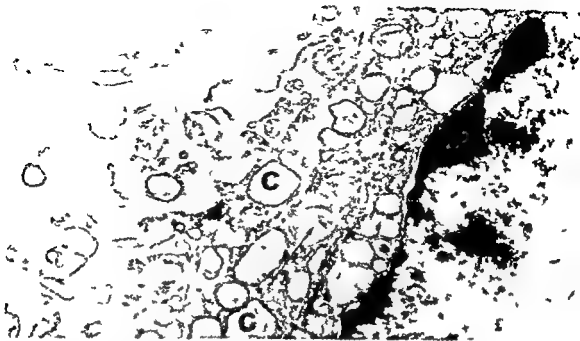


Fig 6 Transmission electron micrograph from the placenta obtained after hypertonic saline abortion. The picture shows a part of the syncytium of a villus in the subchorial zone. Microvilli have already disappeared and the cytoplasm is disintegrating. Vesicles with granular floccular con-

tent probably represent cisternae of the endoplasmic reticulum (c) which are swollen and the nucleus densely clumped chromatin. The mitochondria show only slight damage  $\times 20\,000$

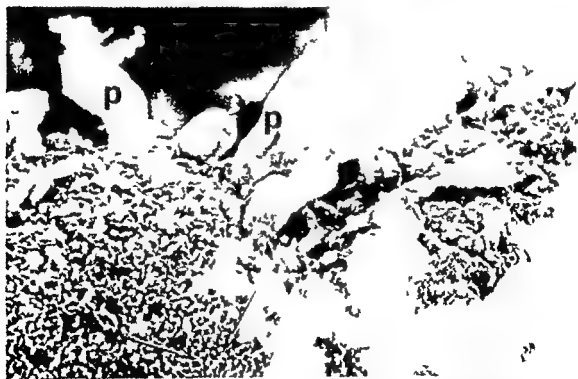


Fig 7 Scanning electron micrograph of the syncytial surface of a placental villus after hypertonic saline abortion. Below on the left microvillous structures still can be identified (ar-

rows) although there are already signs of maturation. On the right no microvilli can be identified and the border of the villus shows many of cytoplasmic protrusions (b, c, d).



8 Electron micrograph of a villus in the decidual region placenta obtained after saline abortion. The difference between the electron-dense syncytium and the not so dense cytoplasm of the cytotrophoblast cell (cyt) is very clear. In 3 layers the mitochondria (arrows) have a normal appearance. Note however the swollen cisternae (c) of the 3 in the syncytial layer and the distorted microvilli. 2 000

**transitional zone** The phenomena observed in the chorionic area can also be found in this region. The disturbances occurred in a milder form however and large ballooning projections which are specific for the subchorionic zone were not observed in the transitional zone although cytoplasmatic protuberances and the matting phenomenon could be found in all of the villi we observed.

**Langhans zone** The matting process was the most characteristic phenomenon in this region. Although the parts of the villi had a rather normal microvillous border large areas were covered with plaques. The other microvilli had pronounced swelling at their tips (Fig 9).

#### *In vitro experiments with hypertonic saline solutions*

The tissue changes observed in HSIA might be the result of autolytic processes due to the fact that the fetus had died some time before the placenta becomes available. To obtain more information we performed the following *in vitro* experiment. From a normal placenta obtained by hysterotomy small samples were placed for ½ hour in solutions of differing salt concentrations. In order to obtain some information about the alterations due to the medium samples were placed for ½ hour in pure isotonic SBSS. The specimens were then subjected to TEM and SEM.

**Results after ½ hour exposure to SBSS** The most striking feature in the TEM pictures were the juxta-nuclear vacuoles (Fig 10). These vacuoles consisted of a local distension of the nuclear envelope. Sometimes vacuoles of this type are described as a common structure in placental anatomy. In our opinion these vacuoles are artefacts due to the period of time that the samples were left unfixed in the medium, as we did not find them in the specimens of the same placenta which were fixed immediately. The cisternae of the endoplasmic reticulum were also a little distended but the microvillous border was intact; this was confirmed by scanning microscopy. The only change we found with the scanning microscope was a slight swelling of the microvillous tips. No signs of matting or cytoplasmic protrusions were found.

**Results after exposure to hypertonic solutions (resp. 2% and 5 per cent NaCl in SBSS)** Exposure to different concentrations of hypertonic saline produced *in vitro* the same alterations as those described for the placentae obtained after HSIA. With TEM disintegration of the syncytial layer and swollen mitochondria in the Langhans layer could be observed (Fig 11). Sometimes the mitochondria and the cytoplasm had become very electron dense, probably due to dehydration. With SEM the same matting was observed as had been found after HSIA (Fig 12). Large areas of the villi were covered by plaques. The tips of the microvilli were swollen and sometimes slight protrusions were present.

The damage observed after *in vitro* exposure to hypertonic solutions was more severe when 5 per cent NaCl solutions were used.

#### DISCUSSION

Our investigations of placental histology after HSIA with light microscopy showed damage in the subchorionic zone and in about 80 per cent of the placental



Fig 9 Scanning electron micrograph of a placental villus of the decidua zone after hypertonic saline abortion in the 16th week of gestation. The left half of the picture shows an area with a rather normal structure although the microvilli

have rather swollen tips (arrows). These areas are rare, however. The right part of the photograph shows a characteristic area with the more common matting (asterisks).  $\times 19\,000$

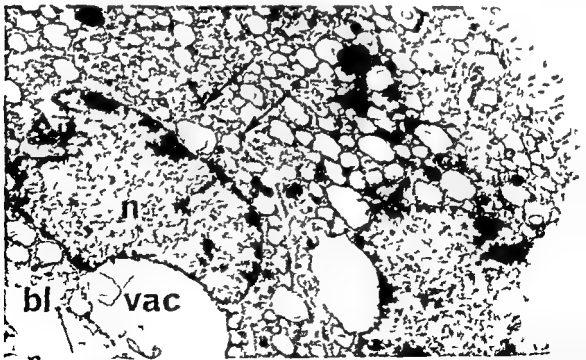
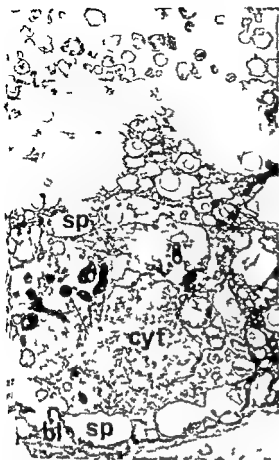


Fig 10 Part of the trophoblast after treatment for  $\frac{1}{2}$  hour in SBSS. The juxtanuclear vacuoles (vac) next to the nucleus (n) are very specific for this experiment. The microvilli show

no changes. Desmosomes (arrow) and the basal lamina are seen. In the basal lamina is a dark spot (arrow) consisting of calcium phosphate deposits.  $\times 10\,000$



11 Transmission electron micrograph of the trophoblast after in vitro treatment with 5 per cent NaCl in SBSS. The microvilli (arrows) in both layers are very electron dense. The cytoplasm of the cytotrophoblast cell shows signs of disintegration  $\times 12,000$

The Langhans layer seems to be much more resistant towards hypertonic solutions than the syncytial layer

Very soon after instillation of hypertonic saline the fetus dies. The changes in the trophoblast however can not be attributed to the death of the fetus. Myers and Panigel (17-19) studied the effects of surgical removal of the fetus on the placenta in the Rhesus monkey. After 5 days the changes in the placenta observed by electron microscopy were very small and quite different from our findings after hypertonic saline. Our in vitro experiments demonstrated that only a very short exposure ( $\frac{1}{2}$  hour) to hypertonic solutions is required to produce impressive histologic changes. The intensity of the damage corresponded to the concentration of NaCl used. The changes produced were similar to those seen in placentae obtained after HSIA. The results of this experiment confirm that the alterations we found in the placenta after HSIA are mostly due to effects produced in the first hour after instillation and are not caused by autolytic processes during the interval between salt instillation and delivery of the placenta.

#### ACKNOWLEDGEMENTS

We wish to thank Drs J H Muller for extensive assistance in preparing the tissue for the scanning electron microscope handling the apparatus and interpreting the micrographs. We are mostly indebted to Mrs Lidia Jongstra Spaapen and Mr J T W A Cornelissen for their technical assistance and enthusiasm in collecting and preparing the material for light and electron transmission microscopy.

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ie a normal structure was observed. These findings are in agreement with other investigations (2, 4, 13). However, electron microscopy showed more widespread damage to the placental trophoblast. In the chorionic zone the changes observed were severe also in the intermediate and decidual zones. Large areas of the syncytium were seriously disturbed. Although a gradual decrease in the damage from subchorionic zone to decidual zone could be observed. At decidual side occasionally some areas of the villi appeared at first sight to be normal. It was revealed on further inspection by transmission electron microscopy however that even in these areas there are changes on a cellular level.



Fig 12 Scanning electron micrograph of part of a placental villus after in vitro treatment with 2½ per cent NaCl in SBSS for ½ hour. Note the microvillous plaque (p) the swelling

ling of the tips of the microvilli (arrows) and the extra protrusions (p) × 19 000

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*Submitted for publication July 7 1978*

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# THE EVOLUTION OF CERVICAL MUCUS INFRASTRUCTURE IN NORMAL CYCLIC BABOONS (PAPIO ANUBIS) AND CASTRATED FEMALES RECEIVING HORMONAL SUPPLIES

## A scanning electron microscope study

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**Abstract** The scanning electron microscope was used to study the evolution of the infrastructure of cervical mucus in normal cyclic baboons and in castrated animals treated with ovarian hormones for establishing an artificial cycle. In both groups the results make conspicuous the progressive enlargement of the filamentous wool which attains a maximum at midcycle and then decreases by degrees in the second part of the cycle. It was shown that the evolution of the framework is very similar during normal and artificial cycle with only variations of slight amplitude. Moreover the variations in the baboon mucus infrastructure closely resemble those described in the human. The results are briefly discussed in the light of known data.

Located at the junction between the vagina and the uterine cavity the cervical canal has a strategic position and constitutes a compulsory passage for sperm ascending from the vaginal seminal pool through the medium of the mucous secretion produced by its upper part. It plays an essential role in the reproductive process. Indeed being easily penetrable during the ovulatory period cervical mucus is known to provide an almost impenetrable barrier to sperms during the major part of the ovarian cycle. The parallelism between changes in some biological properties of cervical mucus and variations in its penetrability by spermatozoa suggested that the phenomenon could be based on a mechanism related at the macromolecular level. That mechanism would modify the mucus infrastructure as a result of hormonal influence.

The physiological importance of cervical mucus and its frequent involvement in human female sterility makes it essential to know its macromolecular infrastructure. The most plausible hypothesis concerning the structure of the solid phase of cervical mucus the so-called tricot like macromolecular gel arrangement was proposed by Odeblad (1968). But although it seemed to provide the most satisfactory

explanation for the rheological properties of cervical mucus as well as its cyclic obstructive characteristic and the changes in its chemical composition during the ovarian cycle Odeblad's scheme required confirmation.

Due to its important depth of field and high power of magnification the scanning electron microscope (SEM) yields a three-dimensional view of the spatial arrangement of cervical mucus infrastructure and presents the tricot like macromolecular arrangement as a matter of fact (1-10). Recently the same technique permitted a study of the variations in cervical mucus framework throughout the ovarian cycle (7) during pregnancy or after menopause (4) and under influence of oral contraceptives (6). Consequently the filamentous three dimensional infrastructure of human cervical mucus appears now to be well established. However the fact that human volunteers cannot be used for experimental purposes makes it necessary to use animals as similar as possible to the human model.

In compliance with the taxonomic proximity of sub-human primates and man preliminary SEM studies have shown recently the striking similarity of human and baboon ovulatory cervical mucus (8).

This study was carried out in order to obtain further insight into changes occurring in baboon cervical mucus during the ovarian cycle and to provide information on the effect of exogenous ovarian hormones on the mucus infrastructure in the castrated baboon female.

## METHODOLOGY

Cervical mucus was obtained from six cyclic adult females (Papio anubis) selected according to three criteria: good health, absence of obvious cervical erosion, satisfactory regularity of the ovarian cycle.



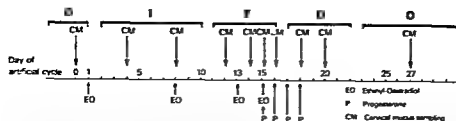


Fig 1 Artificial cycle in castrated female.

To check the ovarian cycle each animal was followed for at least three months with a daily record of the sexual skin. Mucus samples were taken every two days and correlated with the time of ovulation by first day of sexual skin detumescence which occurs in this species on the third day after ovulation (11).

Cervical mucus was also sampled from two females castrated at least 18 months earlier and given subcutaneous injection of 10 mg of Ethinyl-estradiol and 25 mg of progesterone (Luteogyl) according to a sequence that induces an artificial cycle (Table II).

Both cyclic and castrated baboons were anesthetized immediately before sampling using an intramuscular injection of Ketalar at a dose of 0.5 mg per Kg body weight. Cervical mucus was aspirated from the endocervix by means of a Braun probe (1 mm in diameter) adapted to a 20 ml syringe. In order to reduce mechanical stress which could induce distortion of structure in mucus samples the cervical secretion was aspirated very carefully and then evacuated slowly from the probe onto glass coverlips. Only the samples which did not generate bubbles during sampling were prepared for SEM observation. The specimens were immediately plunged into a fixative solution prepared extemporaneously (2.5 per cent of glutaraldehyde in 0.1 M Sørensen's phosphate buffer) rinsed in successive baths of buffer and distilled water then prepared for SEM by freeze-drying according to the process described previously in a technical paper (2). The observation was conducted with a Cambridge Mk II Stereoscan scanning electron microscope under an accelerating voltage of 25 kV.

## RESULTS

**Normal ovarian cycle** The SEM observation of a total of 224 mucus samples demonstrated the striking changes that occur gradually in the macromolecular framework of baboon cervical mucus through the ovarian cycle. At the beginning of the menses period the glycoprotein filaments which constitute the mucus infrastructure appear to form a very compact mesh. From that time the solid wool slackens progressively till the day of ovulation at which time the widening of the meshes reaches a maximum. The reverse phenomenon occurs during the second part of the cycle. The dimension of the meshes decreases by degrees whereas the wool density becomes more and more compact as the luteal phase goes on. This evolution of the mucus framework involves important variations

in both aspect and frequency of structural filaments described previously (1):  
 $\alpha$  filaments 4 000 to 2 000 Å  
 $\beta$  filaments 1 500 to 1 000 Å  
 $\gamma$  filaments 750 to 500 Å

The differences observed appear to be rather constant from one day to the next except in the menses period. Therefore the cycle was divided into periods (Fig 2).

**Menstrual period (Phase +)** During the menses, density of the meshwork seems to depend on the importance of bleeding. While the wool may be very compact in areas containing many blood red cells (Plate I Fig c-d) the filaments are very large and the filaments fairly stretched in other conditions (Plate I fig a b). Filaments constitute the main part of the wool and generally appear to be thick and short and are not distorted by median or lateral thicknesses due probably to the lytic action of menstrual blood. In bloody areas they may be long and smooth without apparent distortion (Plate I Fig b).  $\alpha$  filaments are very rare and  $\gamma$  filaments only occasional. **Period of sexual skin intumescence (Phase I)** At the very beginning of this period which can last to 12 days depending on the animal, cervical mucus exhibits generally a very compact meshwork composed of short and puffed  $\beta$  filaments (Plate II Fig a). The wool tends then to loosen progressively thus inducing a significant increase in the dimension of the meshes (Plate II Fig c-d). Actually the meshwork

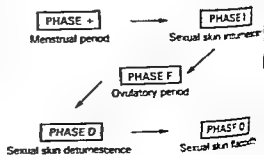


Fig 2

## c 1 Normal cyclic baboons

	Menstrual period	Phase I	Phase F			Phase D	Phase E
			1/3	2/3	3/3		
density	depends on bleeding	+++	++	+	++	+++	+++++
size of filaments							
	+++++	+++++	+++++	+++++	+++++	+++++	+++++
	+	+	++	++	++	+	+
contents of filaments							
ooth		~	+++	+++++	+++	+	
ckled		~	+	++	+	-	
ight	+++	++		depends on constraints			+++
clots	+	+				+	++

vary from 0.5  $\mu\text{m}$  immediately after the menses to 5  $\mu\text{m}$  at the end of the intumescent period. The evolution of the structural filaments is no less significant: the  $\beta$  filaments which are by far the most numerous appear to elongate progressively (Plate II b). Finally they may attain noticeable length (up to 4.5  $\mu\text{m}$ ). At the same time the median thicknesses specific for the previous period tend to grow and their number to decrease whereas thin lateral appendices appear gradually on the  $\beta$  filaments as time passes. By contrast the frequency of  $\alpha$  filaments does not appear to differ much from that of the menses period.

**Period of full development of sexual skin (Phase F)** During the mesh tightening the process of mesh development follows its course until the day of ovulation (Plate III Fig a b). At this time the meshes at their largest dimension which may exceed 12  $\mu\text{m}$ . Concomitantly the surface of the mucus membrane tends to become perfectly smooth and isodiametric along their whole length (Plate IV Fig a b). On the day of ovulation there are no longer any median or lateral nodosities protruding from the  $\beta$  filaments except for occasional appendices which probably correspond to the stem of a broken lateral filaments (Plate IV Fig c-d).

The process begins immediately after ovulation. In the interstices of the meshes delimited by the mucus membrane filaments are seen to tighten progressively while thicknesses and appendices reappear on the  $\beta$  filaments which begin to shorten. The filaments are now more frequent but the frequency of both  $\alpha$  and  $\beta$  filaments appears to be unchanged (Plate III Fig c-d).

As in the human two different three-dimensional arrangements can be observed which seem to be dependent on the physical constraints one can impose on fresh mucus during sampling. When these constraints are sufficiently slight to be counteracted by the thickness and plasticity of the mucoid material lines of force cannot be produced to align the filaments in a particular pattern. The web is therefore similar in appearance to an entangled skein of string and the meshes do not appear as large as in reality. On the other hand when the mucus adheres to the coverslip before it is detached from the probe or if it has been subjected to strong constraint such as an appreciable elongation most of the filaments are oriented so as to be grossly parallel with the lines of force. In that case they may constitute a thin structure that resembles a spider's web and the meshes appear to be larger than in reality.

**Period of sexual skin detumescence (Phase D)** This fourth part of the baboon ovarian cycle has a short duration which may vary between 3 and 5 days depending on the animal. Concomitantly with the increase of sexual skin flaccidity during these few days the process of mesh tightening becomes more and more obvious. At the end of the period the mucus framework appears to attain its highest density (Plate V Fig c-d). In some cases the web is so compact that meshes can scarcely be distinguished. Once more the medium size filaments constitute the major part of the solid phase of cervical mucus: they are always very short and often form bloatednesses of varied size and shape (Plate V Fig b). The dimension of the meshes they delimit rarely exceeds 1  $\mu\text{m}$ . In thin marginal areas submitted to stretching action the

Table II Artificial cycle

	deprivation	Hormonal Phase I	Phase F			Phase D	Phase O
			1/3	2/3	3/3		
Wool density	+++++	+++—	++	+	++	+++—	++++
Types of filaments							
$\alpha$		—	+	+	+	—	
$\beta$	+++++	+++++	+++++	+++++	+++++	+++++	++++
$\gamma$	—	+	++	++	++	+	—
Aspects of filaments							
smooth		+	+++	+++++	+++	+	
buckled			+	++	—	+	
straight	+++++	++		depends on constraints			++++
+ clots	++	+					++

filaments often appear to be puffed up by median distortions and lateral appendicules (Plate V Fig a). The other types of filaments are still rare.

**Period of sexual skin flaccidity (Phase O)** This period which precedes the next menses is the last and also the longest of the baboon ovarian cycle. Its mean duration in our animals was about 10 days. Compared with the former period the sexual skin is now absolutely flat. During phase O the wool density attains its maximum and the compactness of the mesh may be compared with that observed at the end of the menses period (Plate VI Fig b-c d). Consequently the mesh dimension rarely goes beyond 4  $\mu\text{mm}$ .

However the  $\alpha$  filaments are almost absent and the  $\gamma$  type more frequent at the beginning of phase O. In addition the bloatednesses and clots distorting the filaments also appear to be more numerous (Plate V Fig a d). To clarify these results the most significant data concerning the evolution of the solid phase of baboon cervical mucus are summarized in Table I. The effect of exogenous ovarian hormones on cervical mucus framework of castrated females. The results obtained in castrated females treated with exogenous ovarian hormones are very similar to those observed in cyclic animals (Table II). During each period of the artificial cycle the evolution of the structural filaments appears to be grossly parallel to the variations exhibited by the framework of cyclic mucus during the corresponding period.

Indeed with the exception of midcycle during which the glycoprotein filaments do not appear as perfectly smooth in castrated animals the evolution of the mucus framework in cyclic and castrated baboons seems to be so similar that it would be difficult to make a clear distinction.

During the period of hormonal deprivation density of the wool appears to be extreme (Plate Fig a b). The appearance of the framework is similar to that of the end of phase of sexual flaccidity (Phase O) when hormonal supply effective (Plate VIII Fig c d). In both  $\beta$  filaments which constitute the major part of mucus infrastructure during the whole cycle appear to be extremely short and distorted by and terminal nodosities. The meshes they rarely exceed 0.4  $\mu\text{mm}$ . As soon as the third sexual skin intumescence (Phase I) the mesh of the meshes becomes visible in certain areas of samples (Plate VII Fig c d). The mesh widening continues until the middle of phase F (sexual skin development) which appears to correspond to day of ovulation in normal cyclic animals (Plate Fig a b). That phenomenon which induced concomitant elongation and smoothing of filaments will then be reversed so that again extremely compact some days after ending of phase O (Plate VIII Fig c d).

Concerning the mesh dimension and the appearance of the filamentous wool the similarity between the true ovulatory period and the midcycle phase F induced in castrated females appears particularly clear. Indeed with the exception of the median nodosities puffing up the  $\beta$  filaments of ovulatory type, mucus of castrated baboons of medium size filaments appear to have in the almost the same appearance length and size as normal animals. The median nodosities are occasional and the structural filaments exhibit a smooth surface on the major part of their length. The appendicules one can observe here and there on the filaments probably originate from some joining  $\gamma$  filaments.

## DISCUSSION

close resemblance in both appearance and evolution of cervical mucus infrastructure in cyclic baboon in the human is hardly surprising in view of their taxonomic proximity. This similarity which appears to be logical is particularly interesting from an experimental point of view: one has a model as close as possible to the human which in the field of cervical physiology permits many experimental approaches that for evident reasons would not be feasible with human volunteers.

The quasi parallelism in the evolution of baboon cervical mucus framework during normal and artificial cycles is no less important. Being able to produce at will ovulatory and then luteal pictures of the mucus of castrated baboon by injecting diol and progesterone clearly shows that the basis of the observed filamentous infrastructure lies in hormonal stimulation and does not result from mechanical manipulation.

It is not within the scope of this paper to discuss the mechanical aspects of cervical mucus preparation and possible generation of artifacts: the reader who wishes to pursue that subject further is referred to previous studies and discussions that appeared more recently (2, 3, 4, 5). However, some of the data obtained in this study reinforce and confirm my previous results and therefore must be pointed out: the possibility of encountering in dehydrated baboon mucus samples two major spatial arrangements different as the quasi parallel alignment and the folded skein can be accounted for by the plasticity of mucoid material and whether or not a stretching force is applied to it. Since the alignment without breakage of the filaments can be obtained only by stretching the mucus prior to fixation and dehydration, the filamentous structures observed must be considered as evidently pre-existent with regard to the preparative process. Likewise, the mucus plasticity is responsible for local spatial rearrangements resulting in accidental physical constraints: rules out the accurate measurement of important parameters such as number of meshes, mesh dimension and length or number of each type of filament. Therefore, models must be used instead of numbers when accounting for quantitative evolution. The presence of median and to a smaller extent lateral nodosities on the  $\beta$  filaments in the ovulatory mucus of castrated baboon could be due to exogenous progestagenic influence.

The fact that such nodosities and also clots can be observed also in human cervical mucus during pregnancy and after menopause (4) and in the cyclic baboon during the menses period and phase O may be considered to favor that interpretation.

The fact restoring exactly the physiological hormonal sequence in castrated animals can be considered a wager since many parameters interfere some of which cannot be controlled.

Considering the greater length of the baboon spermatozoon compared to that of the human (9) and the similarity of cervical mucus factor in both cases, one might suppose that sperm migration encounters greater mechanical obstacles in the baboon. But such a conclusion must not be inferred: the sperm head has a similar dimension in the two species and the greater dimension of the tail could rather be a facilitating factor.

## ACKNOWLEDGEMENTS

The author wishes to thank Dr A. Psychoyos for permitting the use of his monkeys and C. Olmedo for help with the collection of cervical mucus samples. He is also indebted to Professor M. Bessis for kindly making available the excellent technological facilities of the Institut de Pathologie cellulaire (INSERM U R 48).

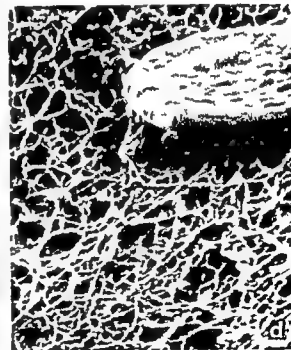
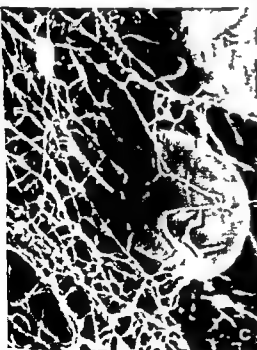
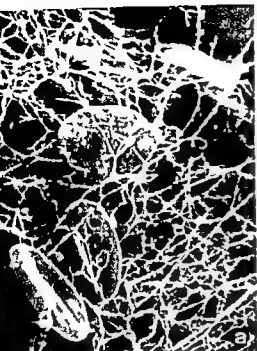
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*Submitted for publication November 9 1977*

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**e 1** Normal cycle Menses period (a) 1st day  
nerous blood red cells are enmeshed in the filamentous  
! which appears to be fairly slackened  $\times 4500$   
1st day Margin of a very bloody sample The filaments  
be distended giving this area the aspect of ovulatory  
meshwork  $\times 4\ 400$

**001** Last day of menses period Despite the presence of some  
red blood cells the wool appears to be very compact The  
filaments are distorted by numerous median nodosities  
 $\times 10\ 000$

**(d)** Last day of menses period Note the extreme density of  
the meshwork at the surface of which a red cell seems to be  
floating  $\times 10\ 000$

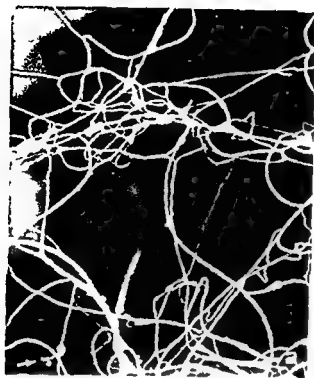


Plate IV Normal cycle Ovulatory period (a b-c-d) The mesh exhibits meshes of very large dimensions. Note the almost perfect smoothness of the filaments and the absence of nodosities. Some broken junctions



filaments may be seen laterally to some filaments (rows). Some clots can be observed (large arrows). Filaments which are exceptional during the ovulatory period where observed in only two females.  $\times 5700$  for a2p

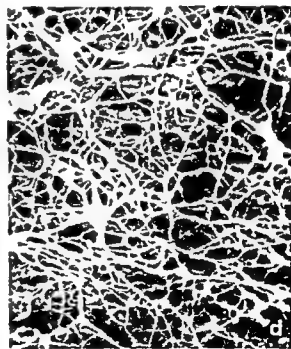
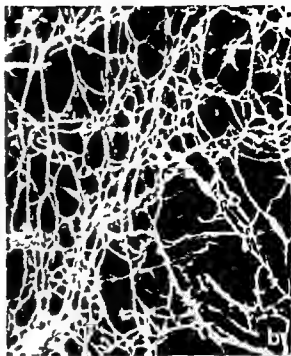


Figure V Normal cycle Phase D (sexual skin detumescence)  
 (a) 2nd day of phase D Marginal area of a sample submitted to accidental traction force Note the clots and deposits puffing up the filaments and the cobweb-like arrangement  $\times 5\,000$

(b) 4th day of phase D  $\times 7\,000$

(c-d) 9th day of phase D Note the tangled aspect of the mucus. A red blood cell is visible in figure c Fig c  $\times 7\,100$   
 Fig d  $\times 5\,400$



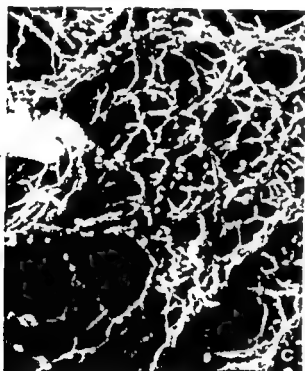
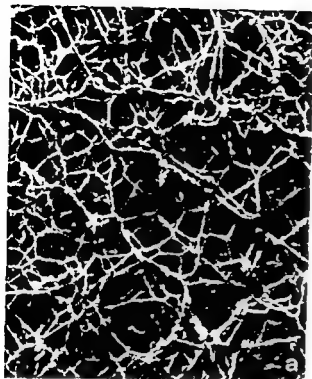


Plate VI Normal cycle Phase II (sexual skin flaccidity)  
(a) 2nd day Clots and nodosities are now frequent  $\times 5\ 000$   
(b) 4th day Note the extreme density of the wool and the

very small mesh dimension  $\times 7\ 850$

(c) middle of phase O (6th day)  $\times 7\ 900$

(d) Last day of phase II (10th day) Some red blood cells and keratinized cells are trapped the wool  $\times 8\ 000$

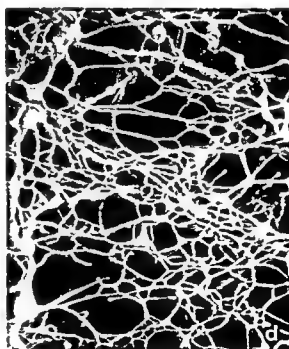
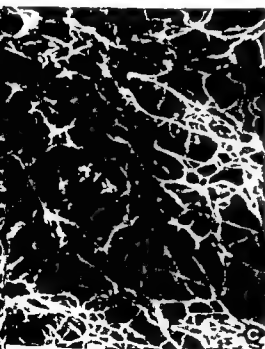


Fig. VII Treated castrated baboon  
 period of hormonal deprivation. Note the extreme den-  
 sity of the wool  $\times 12\ 000$   
 (c) 3rd day of period of sexual skin intumescence. Numerous nodosities  
 are clearly visible  $\times 12\ 000$

(c) 3rd day of period of sexual skin intumescence (phase I)  
 $\times 7\ 200$

(d) 3rd day of phase I. Numerous clots distort the  
 filaments  $\times 7\ 400$



Plate VIII Treated castrated baboon  
(a b) Midcycle period (phase F) Note the ovulatory type arrangement and the presence of nodosities on the filaments  $\times 5\,000$  for both pictures



(c) 2nd day of phase O (sexual skin flaccid) meshwork has tightened considerably  $\times 12\,700$   
(d) 22nd day of phase O The wool is now extremely and compact  $\times 12\,200$

## PERIHEPATITIS IN PELVIC INFLAMMATORY DISEASE — ASSOCIATION WITH INTRAUTERINE CONTRACEPTION

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**Abstract** The Fitz Hugh Curtis syndrome (FHCS) or perihepatitis as a complication of pelvic inflammatory disease (PID) is readily diagnosed by laparoscopy. Among 274 non-selected cases of acute PID verified laparoscopically, FHCS was found in 38 (13.8 per cent). Twenty-four of these patients presented right upper-quadrant pain and tenderness while 14 experienced no local symptoms. Perihepatitis was found in a slightly higher frequency in PID cases of low age and in those using intrauterine contraception (IUCD) but the differences lack statistical significance. However, acute PID occurring within 6 weeks after IUCD insertion was associated significantly more often with FHCS ( $p < 0.01$ ). It is suggested that the traumatizing effect of IUCD insertion facilitates the spread of pelvic inflammatory material to the perihepatic region.

The clinical picture of gonococcal peritonitis of the upper part of the abdomen was first described by Stimson in 1919 (7). In 1930 Curtis (2) reported the frequent association of violin string or band adhesions between the anterior surface of the liver and the ventral portion of the peritoneum with coexistent gonococcal inflammatory disease in the female pelvis. The clinical presentation of this syndrome was characterized by Fitz Hugh in 1934 (3). Most patients present with the sudden onset of right upper quadrant pain and tenderness, occasionally associated with a transiently non-functioning gallbladder, abnormalities of liver function and a perihepatic exudate. Histologically, examination of liver biopsies reveals superficial capsular inflammatory reaction without parenchymatous changes. The aim of the present study was to determine by laparoscopy the frequency of FHCS in a general PID material and possibly relate this to other parameters such as age of the patient and contraceptive practice.

## MATERIAL

The material consists of 274 cases of PID treated in the Gynecological Department, Aker Municipal Hospital, Oslo, in the period January 1, 1976, to Decem-

ber 31, 1977. In all cases the diagnosis was verified by laparoscopy, which also comprised an inspection of the hepatic region. As to clinical appearance, 24 patients complained of right upper-quadrant pain and tenderness of sudden appearance. 14 of them were admitted to hospital within 48 hours. Twenty-one patients were primarily admitted to a non-gynecological department (mostly surgical emergency). The diagnoses on admission are shown in Table I. The pelvic symptoms dominated in only four patients.

## RESULTS

Thirty-eight (13.8 per cent) of the 274 PID cases showed signs of perihepatitis, i.e. patchy fibrine or purulent deposits on liver surface, increased vascularization or hemorrhages in the subdiaphragmatic peritoneum. In four of the cases the changes appeared to be of older date, i.e. violin string or band adhesions, but in these cases inspection of the pelvic organs revealed both acute inflammatory changes and evidence of earlier PID (adhesions).

In 18 patients with PID, perihepatitis was diagnosed laparoscopically without symptoms from this region. Generally, the local reaction on the liver surface in these cases was less pronounced. In most of them a fibinous exudate was observed. The severity of their pelvic affection — as estimated from the degree of visualized inflammatory changes, general symptoms and erythrocyte sedimentation rate — did not differ from those with symptom-bearing perihepatitis.

**Age distribution.** Table II presents the frequency of FHCS related to the number of patients with PID in different age groups. The frequency is somewhat higher in the lower age groups (below 25 years) but the difference is not significant ( $0.05 < p < 0.10$ ).

**Intrauterine contraception.** Of the 120 PID patients using IUCD, 22 (18.3 per cent) showed perihepatitis.

Table I Primary diagnosis on admission to hospital

Diagnosis	No. of patients
Cholecystitis/cholelithiasis	8
Abdominal emergency	7
Appendicitis	4
Liver abscess	1
Pelvic inflammatory disease	4

compared to IE (10.4 per cent) of the 154 PID patients not using IUCD. This difference is not statistically significant ( $0.05 < p < 0.10$ ).

However, when the duration of IUCD use is taken into account (Table III), it is found that the incidence of perihepatitis is significantly higher in the patients who developed PID shortly after IUCD insertion (i.e. within 6 weeks) than in other PID patients using IUCD ( $p < 0.01$ ).

### DISCUSSION

The etiology and pathogenesis of FHCS have not been elucidated. Originally *Neisseria gonorrhoea* was incriminated as being the only etiological agent. Recently the syndrome has been observed in non-gonococcal PID as well (2). Our material cannot resolve this question because bacteriological examination was not performed in all cases. But it seems to us that the role of gonorrhea in the development of perihepatitis has been somewhat overestimated.

The pathogenic agents have been thought to pass from the Fallopian tubes into the paracolic gutters of the abdominal cavity and then to the subphrenic spaces. Recent reports of similar infections in the male (4, 6) suggest other routes of dissemination such as hematogenous or retroperitoneal lymphatic spread.

The Fitz-Hugh-Curtis syndrome is found in 1–10 per cent of cases with PID (2). Tenderness in the hepatic region has been observed in 31 per cent (2). The frequency of the syndrome will depend on the criteria used for the diagnosis of both perihepatitis and PID.

According to modern opinion, laparoscopy is the most reliable diagnostic procedure in cases where PID is suspected (5). We have found laparoscopy superior also in the diagnosis of perihepatitis. The search for perihepatitis has in fact become a routine supplement

Table II Appearance of Fitz-Hugh-Curtis syndrome (FHCS) related to the number of patients with PID in different age groups

Age groups (years)	Frequency of FHCS in PID patients
15–29	15.4 per cent (10/66)
20–24	19.1 per cent (18/97)
25–29	10.9 per cent (7/63)
30–	6.7 per cent (3/46)

to the ordinary laparoscopy in pelvic disease. In the patient in a horizontal position and the scope inserted transumbilically, the upper part of the abdominal cavity is readily visualized.

The presentation of symptoms (right upper quadrant pain and tenderness) seems to be a question of the quantity of the local reaction. Less pronounced perihepatic inflammatory changes are frequently laparoscopically in cases lacking local symptoms.

Weström (8) has reported a higher risk of PID in women using IUCD. Whether FHCS is particularly associated with IUCD is questionable. In our material the syndrome was found in a somewhat higher frequency in the PID patients with IUCD than in those without (18.3 per cent and 10.4 per cent respectively), but the difference lacks statistical significance.

The procedure of IUCD insertion seems to be a factor in the development of perihepatitis. PID appearing shortly after IUCD insertion in the first weeks were associated significantly more often with this syndrome. A possible explanation might be the traumatizing effect of IUCD insertion facilitating the spread of pelvic inflammatory material to the perihepatic region. The present study cannot determine whether this takes place by the lymphohematogenous or direct intraabdominal route.

### ACKNOWLEDGEMENT

The author is grateful to Dr H. Gjønnæss for valuable advice and to colleagues for performing the laparoscopy.

Table III Appearance of FHCS related to time interval from IUCD insertion until PID development

Duration of IUCD use	Frequency of FHCS in PID patients
Less than 6 weeks	42.8 per cent (13/30)
More than 6 weeks	8.2 per cent (7/85)

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*Submitted for publication March 29 1978*

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1981

Date	Place	Name	Office
<b>1980</b>			
May 21	London England	Symposium on Tubal Infertility	Inst OB/GYN Queen Charlotte's Maternity Hospital Goldhawk Road, London W6 0XG England
May 26-28	Viareggio Italy	International Symposium on the Menopause Endocrinological & Pathophysiological Aspects	Serono Symposia Via Ravenna 1 I-00161 Rome Italy
May 27-31	Hamburg Germany	International Congress on Senology	H J Frischbier Universitäts-Frauenklinik Martinistr 52 D-2000 Hamburg 20 Germany
June 9-12	Gothenburg Sweden	XXI Congress of The Nordic Association for Obstetrics and Gynecology	Dr Hans Bergström Dept OB-GYN Sahlgrenska Hospital S-41345 Gothenburg Sweden
June 9-12	Ostend Belgium	Third International Congress on the Menopause	Int. Menopause Society 8 Av Des Bosco B-1150 Brussels Belgium
June 16-20	Florence Italy	XI International Congress of the International Society of Psychoneuroendocrinology	Fondazione Giovanni Lorenzini Via Monte Napoleone 23 I-20121 Milan Italy
June 17-19	Budapest Hungary	International Symposium on Computer aided Diagnosis in Perinatology	Secretariat of the Congress of Computer Aided Diagnosis in Perinatology Mr Z. Katona 1055 Budapest Km. Lajos tér 6-8 Hungary
June 20	London England	Progress in Neonatal Medicine	Symposium Secretary Institute of Obstetrics & Gynaecology Queen Charlotte's Maternity Hospital Goldhawk Road London W6 0XG England
June July 30-2	L. Aquila Italy	International Symposium on Oligozoospermia	G. Fratesi Clin Med V-Polichino Umberto I-00100 Roma Italy
June July 30-3	Leuven Belgium	Gynecological microsurgery A practical course	Dr W. Boeckx Centre for Microsurgery Academic Hospital St Rafael 300, Leuven Belgium
July 1-5	Bordeaux France	International Symposium on IUD Technology	Dr Karl-Gösta Nygren University Hospital S-75014 Uppsala Sweden
July 4-6	Toulouse France	6th International Symposium on Sex Education	C I F R E S 17 Rue de Nimes, 31400 Toulouse France
July 5-11	Madrid Spain	Xth World Congress on Fertility and Sterility	Congress Secretariat Calle San Bernardo 5 Madrid Spain
July 4-8	Rome Italy	International Congress on Women and Sport	Scientific Secretariat International Congress on Women and Sport Piazza Mignanelli 4 00187 Rome, Italy
July 8-11	Edinburgh England	22nd British Congress of Obstetrics and Gynaecology	Royal College of Obstetricians & Gynaecologists 27 Sussex Place Regent's Park London NW1 4RG England
July 20-23	Williamsburg USA	AAGL Laparoscopic sterilization A comparison of the methods	American Association of Gynecologic Laparoscopists 11239 South Lake Dr Downey California 90241 USA

## VAGINAL YEASTS IN PARTURIENTS AND INFESTATION OF THE NEWBORNS

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act Vaginal yeasts from 992 full term pregnancy parturients in the delivery room were cultured and identified. The yeasts were similarly cultured from the mouth of the infants seven days after birth to find out whether a viable infection had taken place. Anamnestic data concerning maternal infection earlier or during the present pregnancy were collected from the parturients. The yeast findings are compared with results from similar cultures from gynecological outpatients.

Vaginal yeast infection had been diagnosed before the present pregnancy in 16.5 per cent and in some phase of the present pregnancy before the delivery in 8.5 per cent of the parturients. A positive yeast culture with no clinical symptoms during the pregnancy was found in 9.9 per cent. Maternal symptoms were present in 61 per cent of the yeast positive parturients.

Together 3 155 specimens were studied and 670 yeasts identified. Positive yeast cultures from parturients were obtained in 23.7 per cent and from gynecological patients in 30.9 per cent. *Candida albicans* was the most common yeast. It was followed by *Saccharomyces cerevisiae* in parturients and *Torulopsis glabrata* in the gynecological patients. *Pityrosporum ovale/orbiculare* was also common in the latter material. *S. cerevisiae* as well as *Pityrosporum* spp. are usually not identified in the medical yeast laboratories for methodological reasons.

A positive yeast culture from the mouth of the newborns was obtained only in 20 cases. A symptomatic oral candidiasis was not present in any of the cases.

Reports vary concerning the frequency of vaginal candida infection during pregnancy. The figures usually range between 17 and 50 per cent depending on the methods used and the kind of material studied. Findings reported during the last few years have been compiled in many papers (5, 6, 8, 16). Similarly there are variations in the reported frequencies of symptoms due to candida infection (candidosis or candidiasis). The most common figures state that 1/2 of the patients are symptomless. Comparative studies on fungus infestation in mother and child have shown unequivocally that the infected birth child represents the principal source of infection for

the infant. Smidt (20) and Holtorff *et al.* (10) discovered vaginal candidiasis in the mother in 85 per cent of newborn babies with yeast infections. Careful studies by Blaschke-Helmessen (1) showed that *Candida albicans* strains detected simultaneously in the mother and child are culturally identical. While all investigators agree that *Candida albicans* is the most common yeast isolated from vagina, there are considerable differences in the reported frequencies of other yeasts. For example *Torulopsis glabrata* is hardly mentioned by some investigators while it comprises up to 30 per cent in other studies (24).

The purpose of the present study was to determine the frequency of candida in the vagina of full term pregnancy women admitted to delivery room in our Central Hospital. The yeasts have been cultured in order to identify the infesting yeast species. Yeast cultures have also been carried out from the oral cavity of the newborns one week after birth in order to find out to what extent viable transfer of yeasts takes place during birth. The frequency of the various yeast species in the vaginal samples are compared with results collected from gynecological outpatients of our hospital.

## MATERIAL AND METHODS

The number of mothers was 992. The youngest mother was 16 and the oldest 45 years-old, most belonged to the age group 25-29 years. They were carefully asked about episodes of vaginal discharge and vulval symptoms, possible medication used etc. Samples were taken from the middle part of the vaginal wall after the women had been admitted to the delivery room at the beginning of labor. The samples were taken with loop directly to fungal culture medium (Dixon's yeast medium) was used and the culture was carried out immediately at 37°C for three to seven days. Samples were collected in the period 30.12.1977-8.5.1978 at our University Central Hospital.

Samples were similarly collected from the mouth mucous membrane of the infants seven days after delivery while still in hospital. Cultures were made immediately in the



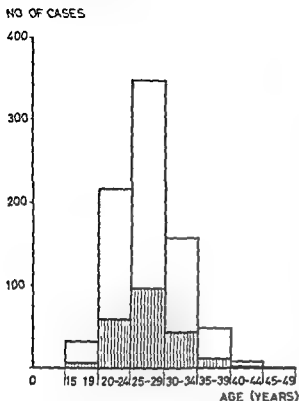


Fig 1 White columns: age distribution of all parturients  
Dark columns: age distribution of yeast positive

medium in exactly the same way as from the mothers. Identification of the yeast was carried out according to Lodder (13).

## RESULTS

Medically treated vaginal discharge and/or vulval symptoms at some time earlier was registered anamnesticly in 23.9 per cent and during the present pregnancy before delivery in 12.2 per cent. A diagnosed vaginal yeast infection was registered some time before the present pregnancy in 16.5 per cent and during it in 8.5 per cent. These patients had had typical discharge, redness and genital pruritus which had led to a medical consultation.

The yeast cultures of the parturients taken in the delivery room were negative in 756 and positive in 236 cases, i.e. a positive culture was found in 23.7 per cent of the total of 992 parturients. In 61.0 per cent of these there had been symptoms during the pregnancy. A positive yeast culture with no clinical symptoms during the pregnancy was found in 9.9 per cent of the parturients. The yeast species identified are listed in Table I. As expected, the most common isolated

species was *Candida albicans*. The high frequency of *Saccharomyces cerevisiae* is noteworthy.

A yeast culture was obtained from 821/801 of them turned out to be negative. Thus, positive cultures or less than three per cent amount (Table I). Again *C. albicans* was the most common species identified. In six of these *C.* cases the culture from the mother's vagina was negative, which means that the source of yeast mother's vagina. Clinical signs of oral were not found in any of the infants at the time of sampling.

The age distribution for parturients with vaginal yeast culture was similar to that for all parturients as seen in Fig 1.

For comparison Table I also includes our yeast finding in gynecologic patients from the outpatient department of the same hospital. It is seen that a positive culture was obtained from 10 per cent of the cases. The most common was *C. albicans* but the high frequency of *T. candida* compared to the few findings of this species in parturients is to be noted. Another finding is the relatively high frequency of *P. ovale* (including *ovale* and *orbiculare*) in the patients compared to the parturients.

## DISCUSSION

The method of collecting specimens for culture media and carrying out the examination immediately can be expected to yield the most data about the frequency of vaginal yeast infection. It was shown, for instance, by Timonen (14) that in a material of 10 344 patients only one seventh of cases of mycosis can be detected by preparations made by Papanicolaou's stain, even when special attention is paid to yeast cultures. Holmberg and Laudanska (9) found 10 per cent of cytology material from a health control survey to be positive. According to Thomsen-Pedersen (23) microscopy of the vaginal samples seldom revealed a diagnosis with or without PAS-staining and addition of potassium hydroxide. A 10 per cent concentration led to suspicion of candidiasis in only about half of the cases. Methods like these have been used in some of the earlier studies without cultural verifications, which may explain some of the great variations in the reported

Table I Yeasts identified in vaginal samples from parturients and in the mouth mucosa of their infants one week later. For comparison are given yeast findings in gynecologic patients from the same hospital

Identified	Present study		Gynecologic patients	Total
	Mother	Infant		
<i>Candida albicans</i>	171	15	263	449
<i>Candida guilliermondii</i>	1	0	0	1
<i>C. krusei</i>	11	0	10	11
<i>Candida parapsilosis</i>	1	1	9	11
<i>C. rugosa</i>	0	0	1	1
<i>Candida tropicalis</i>	0	0	2	2
<i>C. m.</i>	3	4	50	57
<i>Torulopsis</i> sp.	0	0	1	1
<i>Saccharomyces cerevisiae</i>	52	0	25	77
<i>C. glabrata</i>	7	0	11	60
<i>Sporoporum cutaneum</i>	1	0	0	1
Total	236	20	414	670
Culture negative	756	801	928	2 485
Total	992	821	1 342	3 155

Stauramo and Penttinen (17) using yeast cultures found mycosis in 23 per cent of pregnant women. They found mycosis in 25 per cent of gynecologic patients with vaginal discharge. In a study of gynecologic patients Timonen *et al* (24) found vaginal yeast infection in 35.5 per cent. Varonen and Tervilä (25) found vaginal mycosis in 28 per cent of primiparae during the time of delivery. Finding of 23.7 per cent in parturients at the time of delivery and 30.9 per cent in the gynecologic patients are well in line with these results. This suggests the prevalence of candidosis during pregnancy has not increased markedly during the past decade or so in this part of the world. This would agree with Moberg and Laudanska (9) who found no proof of a general increase in candidosis in a Swedish population.

From other parts of the world the frequency of candidosis for vaginal candida infection during the last trimester of pregnancy is often cited (5, 16). The figure has remained at the same level for years, although still higher figures have been reported. The frequency of positive cultures rises with the duration of gestation. Emur (4) found occurrences of 11.4 per cent, 13.3 per cent and 36.4 per cent for candida infections in the 1st, 2nd and 3rd trimester of pregnancy respectively. In nonpregnant women the prevalence of vaginal candida infection is usually stated to be around 11.13 per cent (19). Higher figures are reported for users of contraceptive pills. For normal populations outside venereological clinics, however, the real prevalence remains below that found during the last trimester of pregnancy (15).

About one third to one half of those carrying candida infection are usually reported to suffer from vulvo-vaginal symptoms. In our material about two thirds of the women with yeasts were considered symptomatic cases. It is however to be noted that this refers to the symptoms throughout the pregnancy and not just those of the last few days or even of the last trimester alone.

The age curve of the patients with a positive vaginal yeast culture was similar to that of the whole parturient material. This shows that yeast infections are not prone to any particular age group. Also Clark and Solomons (2) have shown that there is no definite variation in the frequency of candida vaginitis during the reproductive phase. It has further been found that the incidence of vaginal mycosis is not related to age or parity (3, 7, 11).

The yeast species identified by us appear to be the same as those reported in most of the earlier studies, *C. albicans* being the most common. The remarkable thing in our findings from the end of pregnancy is the small number of different species found in comparison to the gynecologic patients. *Torulopsis glabrata* was seldom found among the parturients, whereas it represents 13 per cent of the yeasts in the gynecologic material. In some studies its frequency has been found to be even higher in the Finnish material of Timonen *et al* (24) it represented 31 per cent. Several other reports hardly mention it. This suggests that among gynecologic cases *T. glabrata* is to be expected in a high frequency, while in obstetric materials it is rare. Whether this is due to special environmental vaginal conditions during pregnancy re-

mains to be seen

Another noteworthy finding is the high frequency of *Saccharomyces cerevisiae*. This species is also seldom mentioned in reports on genital mycoses and its role in causing genital symptoms is disputed: some state that it is a commensal, others that it is a pathogen. The fact that it is often not mentioned among the yeast species found is clearly a consequence of the methods used. It is easily overlooked as a candida species other than *C. albicans* unless adequate fermentation and assimilation series are done and ascospores are searched for after adequate stainings. This is seldom undertaken in medical mycological laboratories. The fairly common finding of *S. cerevisiae* in symptomless patients suggests that it has no pathogenic role. However, one occasionally meets patients who have recurrent genital symptoms and repeatedly only *S. cerevisiae* can be identified in cultures. In this material *S. cerevisiae* appeared to be somewhat more frequent in the parturients than in the gynecologic patients.

Another species which has hardly been mentioned in reports on genital yeasts is *Pityrosporum ovale* and *orbiculare*, which many consider to be a single species. This is due to the fact that *Pityrosporum* does not grow on the usual media for yeast culture. We used Dixon's medium, which allows growth of *Pityrosporum*, and found it quite frequently in vaginal cultures. Whether it is to any extent a pathogen is unknown.

The yeast infected birth canal is stated to be the most usual site of infection for the newborn. In some works this has been the subject of special studies and even 85 per cent of the infants born to yeast carriers have been claimed to be infected during birth (1). Much lower figures have, however, also been reported. In our study the specimens from the mouth mucosa were collected 7 days after delivery with a view to determining the frequency of oral infection that was stable enough to cause candidosis. In several other studies specimens have been taken immediately after birth, when the mouth has certainly contained transient vaginal secretion which is no doubt eliminated very rapidly. Consequently our finding of positive candida cultures from the mouth specimens was very small, about 3 per cent. And even these include several cases where the mother's vaginal culture was negative, so that the source of infection was not her birth canal. Other possible sources of yeast infection could be e.g. the mother's mouth, since the mouth of adults of that age is yeast infected in some

25 per cent. Other sources could be the mouths of the nurses, doctors and others who attend the babies. Air, food, cloth, bottles and utensils may be minor contributors.

According to Lachenicht *et al.* (12) about 10% of the babies infected during birth later develop oral candidosis. It remains to be found in further studies whether this holds true in the present material. Intertriginous cutaneous candidosis is the other relatively frequent consequence of candida infection. Bronchopulmonary mycoses due to aspiration of yeasts and infestation of the gastrointestinal tract followed by digestive disturbances are not so rare, while more serious types of infection usually occur only in connection with immunological deficiency states.

The symptoms of candidosis appear very soon in the newborns of the yeast infected mothers, candidosis which requires treatment can be already on the fourth post partum day (10). 10% of our subjects could, however, a clinically oral candidosis be detected. Any case of oral candidosis in the newborn is, however, often requires treatment. In order to eliminate the most frequent site of infection, it has been suggested that a vaginal yeast culture should be taken in the gravida before the calculated delivery time (14, 21, 22). This is not done at present in Finland. In contrary opinions about its necessity are presented. The same applies to the nystatin, the axis of all newborns which some authors have requested (12).

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Submitted for publication June 6 1978

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1981

Date	Place	Name	Office
<b>1980</b>			
September 2-3	Barcelona Spain	Congreso Europeo Perinatal	VII Congreso Europeo de Medicina Perinatal Apartado de Correos 129015 Barcelona Spain
September 2-5	Barcelona Spain	7th European Congress of Perinatal Medicine	Congress secretariat Apt 29015 Barcelona Spain
September 2-6	Berlin Germany	6th International Congress of Psychosomatic Obstetrics and Gynecology	Ass Prof Dr M Stauber F-1000 Charlottenburg der FUB Pulstr D-1000 Berlin 10 W Germany
September 4-7	Kiwah Island Charleston SC	International Symposium on Carcinoma of the Cervix Biology Etiology & Diagnosis	E S E Hafez M D OB/GYN State University Medical Res Bldg 550 E Canfield Detroit MI 48201
September 15-16	Bologna Italy	First International Symposium on Recent Advances in Prenatal Diagnosis	A C Assistenza Congressi Via L. Palagi 21-40138 Bologna Italy
September 22-28	Varna Bulgaria	3rd International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Aging	Bulgarian Academy of Sciences J G Vassileva Popova c/o Dept of Biophysics 113 Sofia Bulgaria
September 24-27	Kiel West Germany	Embryo Transfer and Instrumental Insemination	Professor Kurt Semm Abteihus Frauenheilkunde D-2300 Kiel 1 W Germany
Sept-Oct 29-1	Freiburg Germany	International Congress on Endocrinology of Human Infertility	C Ferrari M D P O Box 995 Milan Italy
September 30	London England	Anti androgen therapy for hirsutism	Symposium Secretary Inst of Gynaecol Queen Charlotte Hospital Goldhawk Road W6 0XG England
October 3-5	New Delhi India	3rd International Seminar on Maternal and Perinatal Mortality Pregnancy Termination and Sterilization	Hon General Secretary The Fed Obstetric & Gynecological Soc India Purandare Gnha 31/c Dr N A Purandare Marg Bombay 400 007 India
November 14-17	Madrid Spain	7 Congrès Européen de Médecine Périnatale	Professor de la Fuente Maternidad de la Ciudad Av Generalísimo 177 Madrid 28
November 18-23	New Orleans Louisiana USA	Clinical Symposium on Gynecologic Endoscopy	American Association of Laparoscopists 11239 South 1st Boulevard Downey California 90241
November 20-22	Barcelona Spain	Symposium Internacional Sobre Monitorización Prenatal	Instituto Dexeus Srtas M Lluís Ana Baldrich c/Paseo de Gràcia 17 Barcelona 17 Spain
December 1-4	Kairo Egypt	Second Congress of the International Society for the Study of Hypertension in Pregnancy	Docent Hjördis Robbe Dept of Obstet and Gynecol Karolinska Hospital S-104 01 Stockholm 60 Sweden
December 1-12	Melbourne Australia	Seventh UICC Training Course in Cancer Research	Dr A W Burgess UICC Centre The Walter and Eliza Hall Institute Royal Melbourne Hosp P O Box 120 Victoria Australia

## ETIOLOGY OF PREMALIGNANT CERVICAL LESIONS IN TEENAGERS

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**Abstract** The etiology of premalignant cervical lesions was anamnestically in teenagers. The material comprised of 54 patients (mean age 18.0 years, range 12-19 years) treated in 1973-77. Early coital practise and frequent change of sexual partners were usual. Previous abortions and frequent genital infections were also typical. The high frequency of dysplasias in young age groups calls for general knowledge and information even among teenagers and if possible mass screening.

In 1974 we reported an increase of premalignant lesions since 1968 in teenagers (8-9). Infection seemed to be the cause but it was not possible to prove that it was definitely responsible. Since then the increase appears to have continued. Table I and Fig 1 show that it could be the cause of this tendency?

## MATERIAL AND METHODS

In order to clarify this problem anamnestically. A careful medical history was taken from 54 patients aged  $\leq 19$  years treated in 1973-77. The diagnosis of moderate or severe dysplasia was based on histopathological examination at the Department of Obstetrics and Gynaecology, Turku University Central Hospital.

## RESULTS

The mean age of the patients was 18.0 years (range 12-19 years). Eleven patients were married. Twenty-two patients were students, twenty belonged to the group of skilled labor or junior office employees, seven were unskilled workers and five were higher office employees.

The mean age of menarche was 12.7 years (range 10-16 years). Thirty-six patients had undergone previous gynecological examination at least once. Vaginal smear had been taken from 24 patients. Three patients had had gonorrhea, 36 patients simple vulvovaginitis and five patients pelvic infection in their medical history.

Coital practise had started at the age of 14.2 on average (range 11-18 years). The patients had had six partners on average (range 1-100, four of them had had ten partners, two patients 20 and one patient over 100 partners). Coital frequency varied between three times a year and many times a day, the most common being twice a week.

Six patients had never used any preventive method. Forty patients relied on condoms. Pills were used by 28 patients (16 used mini pills, 12 had combined pills), two patients used foam, one patient had an IUD. The duration of use varied between two months and several years. Ten patients had had one delivery.

Table I. Premalignant cervical lesions in teenagers in the Department of Obstetrics and Gynecology, Turku University.

	Total amount of dysplasias	Dysplasias at age $\leq 19$ years	
		No. of cases	Per cent
12-69	85	4	4.7
70-71	130	3	2.3
72-73	127	16	12.6 ( $p < 0.01$ )
74-75	68	15	22.0
76-77	89	35	39.3
Total	499	73	14.6

Fig 1

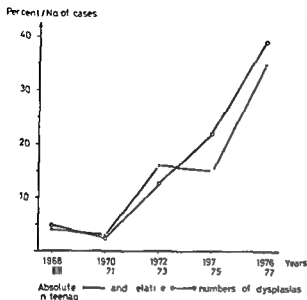


Fig 1 The rising frequency of dysplasias in young age groups

two patients two deliveries one patient had undergone two spontaneous abortions and a legal abortion had been performed in ten patients

### DISCUSSION

An increase of premalignant cervical lesions in teenagers has been verified by several authors (1, 2, 3, 8, 9). Two etiological theories have been presented: early coital practise and frequent change of sexual partners. In our material the mean age at the first sexual intercourse was 14.2 years, while the usual rates among the Finnish female population are 19.3 years in women born in 1942–46, 18.8 years in those born in 1947–50 and 18.1 years in those born in 1951–53 (10). The decrease in age at first sexual intercourse was distinctly faster than the decrease in the age of sexual maturity. Considering the young age of the patients in our material, we must also pay attention to the number of sexual partners (6 on the average). Sievers *et al.* (10) have reported that 4 per cent of the unmarried Finnish women in age group 18–29 years had had  $\geq 10$  sexual partners and 16 per cent had had 4–9 partners.

Two clinical factors are present in the vast majority of young patients with cervical premalignant lesions: sexual promiscuity and contraceptive pills (5). In our

own material more than half of the patients use pills. Therefore it is understandable that they have higher sexual activity and higher incidence of cervical ectopy, as shown by Moghissi (6). According to Leppäluoto (4) it may be inferred that both in promiscuous young women and in young women using the pill the increased incidence of cervical metaplasia, ectopy plus the active sex life are likely to increase the possibility of a chance hit between coitus-associated carcinogen and the target cells. Clinically an increased number of women with suspicious Papanicolaou smear and with 'surface dysplasia' would be expected to emerge from the two groups of young women.

Previous abortions and frequent genital infections of various types were typical in our material. In earlier material the frequency of cytomegalovirus antibodies was the same as in a healthy population. The frequency was a little greater in our material, but the difference was not statistically significant (9). On the other hand, in patients with cervical cancer we have found distinctly increased amounts of H V H 2 antibodies (7). The possible role of infection as a causative factor, however, needs further study.

The Scandinavian Association of Obstetrics and Gynecologists (11) recommends that the mass screening programs should be continued and especially at risk groups. However, because the incidence of cervical carcinoma in the age group 15–24 has significantly decreased at the same time as the number of dysplasias in younger age groups has increased, we should seriously try to increase our knowledge and information in the latter aspect and ensure that the mass screenings reach them. We definitely agree with Leppäluoto (4) who states that the mass screening should be continued.

From a clinical point of view, the restoration of healthy cervical epithelium and a healthy vagina would serve as a prophylaxis for premalignancy.

### ACKNOWLEDGEMENT

This work was supported by a grant from the Lars Armas Koivurinta Foundation.

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 javik June 1-4 1976

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## CASE REPORT

PRENATAL DIAGNOSIS OF OMPHALOCELE BY ULTRASOUND  
AND AMNIOGRAPHY

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omphalocele is a herniation of viscera into the base of the umbilical cord with a covering membranous sac of peritoneum amnion as opposed to umbilical hernia which is covered by skin (9). The umbilical sac is insulated into the sac (6-11). Omphalocele presents a failure in the normal developmental process before the tenth week. The incidence varies from one of 3 200 to 10 000 live born infants (3-9). Associated anomalies vary in reported series from 0-10 per cent or more. In a survey of 236 cases 10 per cent (10) found 18 per cent. The malformations are usually intestinal defects but non jejunoileal in contrast to malformations in gastroschisis (10). Most patients have incomplete rotation and fixation of the gut (9-10). Severe cardiac malformations are frequent (Moore (10) 20 per cent) and omphalocele often plays a major part in specific syndromes (9). Unfortunately but not all infants are premature. Chromosomal abnormalities seem to occur with increased frequency (11). Lately reports have linked maternal serum and serum fluid alpha fetoprotein elevation with fetal omphalocele (1-2 4 7 13 14) and polyhydramnios is almost invariably related to the presence of the malformation (8-14). Ordinary radiographs rarely reveal soft tissue elements while amniography is a most useful tool to detect fetal malformations. Within the last year reports of intrauterine diagnosis of omphalocele by ultrasonography have been published (3-4 5). This paper is a report of an omphalocele diagnosed prenatally with ultrasound scanning and amniography with concurrent values of plasma estradiol and amniotic placental lactogenic hormones recorded. Maternal serum and amniotic fluid alpha fetoprotein values were measured and chromosome analysis was performed.

## CASE HISTORY

The mother a 33 year old woman gravida V para IV had no previous medical history or familial dispositions to malformations. She had slight vaginal bleeding and contractions in the 11th 18th and 19th weeks of the pregnancy.



Fig 1 1 Intestinal loops inside the omphalocele at the umbilical level  
2 Omphalocele long axis 9 cm  
3 Amniotic fluid



Fig 2 Amniography. A p (Fig 2a) and lateral (Fig 2b) views with corresponding drawings of all visible surface structures of the female fetus. Radiographs taken 24 hours after the injection of meglumine amidotrizoate (Urografin) 76 per cent 15 ml and lipiodol ethylique fluid (Lipiodol) 6 ml in to the amnion. The water soluble Urografin is only visible in the fetal intestines (small arrows) while the only lipiodol outlines the vernix coated fetal skin as a thin radiopaque



film. There is no visible contrast medium on the surface of the abdomen indicating either lack of coated skin or a non visible soft tissue process lying in contact with the abdominal surface. The position of the lowermost hand of the fetus (Fig 2a, big arrow) indicate that it rests on such a soft tissue process. The direct signs were interpreted as a big omphalocele, indicating no intestinal structures at the time of examination.

Two ultrasound scanings in weeks 11 and 19 showed no sign of a placenta previa or anything abnormal. In week 34 polyhydramnios was obvious. The uterus was equivalent to week 32, she had gained 15.8 kg in weight and her blood pressure was 135/90-160/110. She had light edemas. The plasma alpha fetoprotein value was above normal. She was treated with hydroflumethiazidum (ROXYL<sup>®</sup>) and the edemas disappeared but polyhydramnios persisted. A new ultrasound scanning revealed a well-defined omphalocele (Fig 1) and a fetus with a biparietal diameter of 7.3 cm. An amniography was performed (Figs 2a and 2b) and supported the diagnosis of omphalocele but did not reveal any other malformations. Amniotic fluid alpha fetoprotein, however, was significantly increased compared to our normal standard. In order to prevent pre term labor the mother was treated with ritodrine chloridum (UTOPAR<sup>®</sup>) intravenously but 5 days later because of slight contractions she was transferred to the Department of Obstetrics at Rugehospitalet since the Herlev Hospital does not have a neonatal intensive care unit and division of neonatal surgery. At this time four consecutive values of plasma es-

tradiol and plasma placental lactogenic hormone within normal ranges.

Two plasma alpha fetoprotein values were further increased. A final ultrasound scanning estimated the fetus to 39 weeks (biparietal diameter 7.7 cm). One mild contraction reoccurred in spite of maximum dose UTOPAR<sup>®</sup>. The mother was given Betametasone phosphate (CELESTON<sup>®</sup>) 12 mg intramuscularly to prevent respiratory distress syndrome in the newborn. Approximately 18 hours later a cesarean section was formed.

A live girl, weight 2025 grams, was delivered. She had an Apgar score of 3 after 1 minute and 6 after 5 minutes. She had respiratory acidosis and after 10 minutes was put to a respirator in which she recovered fairly well. Antenatal treatment was initiated at delivery. A congenital omphalocele with amniotic covering protruded through the umbilical ring 2 1/2 cm in diameter (Fig 3). A few loops were easily reposed into the abdomen and the ligament at the base and extirpated. The remaining defect was covered with plaster.



as than 24 hours later the infant died because of cardiac arrest with low blood pressure. During these last hours the abdominal volume increased considerably and puncture of the abdominal wall showed peritoneum. An intravenous urography showed no excretion from the kidneys. Autopsy diagnoses were: Severe hemorrhage of the suprarenal glands, pancreatic hyperplasia, renal infarction, subarachnoidal petechiae over the hemispheres and under the cerebral basis. There were no signs of malrotation of the intestines. The heart was very small but otherwise normal. The main vessels were normal. The lungs contained little air and were voluminous.

## DISCUSSION

This case history illustrates developments in the prenatal diagnosis of severe malformations by using sophisticated techniques in ultrasonography and radiography. Close cooperation between the obstetric department, the ultrasound laboratory and the X-ray department should provide obstetricians with much more possibilities of predicting severe or lethal conditions in the fetus, thus yielding the options of either terminating a pregnancy before term in order to avoid delivery of an infant with such severe malformations that the chances of survival are nil or

preparing for a delivery under optimal conditions so as to improve the infants chances of survival without further risks evolving from little knowledge of the prenatal intra uterine situation. It also demonstrates however that even with a well substantiated set of information it is extremely difficult to predict the outcome if a high risk pregnancy is allowed to continue.

It could be argued that the risk of associated anomalies to omphalocele is so great (3, 4, 8, 14) that if the diagnosis is made prior to week 28 or if possible week 20 as achieved by Campbell *et al.* (4) an abortion should be performed so as to spare the parents the tragedy of a child with malformations incompatible with survival for more than a short time.

The development of severe hydrops should always induce a thorough examination by a highly skilled ultrasound technician or an amniography.

Diabetes and RH immunization should be ruled out.

From our case it seems that plasma estradiol and plasma placenta lactogenic hormone values do not give any indication of the diagnosis and although reports (1, 2, 4, 7, 13, 14) of elevations of serum and amniotic fluid alpha fetoprotein in concordance with



Fig 3 Infant immediately after delivery

omphalocele have been published lately the available information is so sparse that it only serves to warn the obstetrician of omphalocele as one of the many serious prenatal conditions associated with this symptom.

If however the omphalocele is diagnosed so late in the pregnancy that abortion is out of the question the utmost precautions should be taken to prolong the pregnancy until term in order to prevent the delivery of a premature child. It has been shown (10) that 59 per cent are born prematurely or have low birth weights and the prevention of respiratory distress syndrome by prolonging the pregnancy administering betametasone to the mother and delivering by caesarean section should improve the newborn's chances of survival. The infant must be warded in an intensive-care department with a neonatal surgery unit and the proper operative technique applied if repair of the hernia is absolutely necessary. Here especially it is recommended to avoid opening the amniotic covering since this poses grave risk of sepsis (9, 10). The reposition of a large hernial content into the abdominal cavity involves the risk of pushing the diaphragm high up into the thorax which will augment respiratory distress (12). In addition the increased content of the abdomen might create a compression of the inferior vena cava with blockage in the venous return diminished cardiac output and eventually shock and cardiac arrest.

Other complications of overcrowding the abdomen are impaired kidney function and intestinal obstructions with pressure necrosis. In all it seems nonoperative management of intact omphalocele especially valuable in premature infants with a peritoneal sac (9).

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Submitted for publication March 2 1979

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## CASE REPORT

## RECURRENT URINARY INCONTINENCE TREATED NEUROSURGICALLY

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Neurogenic bladder dysfunction can be difficult to manage and is usually impossible to cure. This case report describes neurosurgical treatment of a case of recurrent incontinence in a 56 years old woman who was previously treated on four occasions with vaginal repair operations with no beneficial effect.

Cystometry revealed detrusor hyperreflexia (supranuclear bladder paresis). Myelography demonstrated cervical spinal cord compression. She was treated with spondylodesis of the cervical spine with complete relief of incontinence. 18 months postoperative cystometry was normal and after 2 years she was free of symptoms.

Neurogenic bladder dysfunction is a known sign and symptom of many diseases such as multiple sclerosis, Parkinson's disease (3), cerebral vascular insults and spinal cord lesions (4).

Supranuclear bladder paresis is mainly treated with parasympatholytic drugs (10), sacral nerve blocks (9) or sacral rhizotomy (8) with improvement in 50 to 75 per cent of the patients. However, the treatment of this bladder disturbance is mostly palliative.

Only in a few cases is causal treatment of the primary neurological lesion possible. This report describes such a case.

## CASE REPORT

In 1975 a 56 years old woman with recurrent urinary incontinence was referred for urodynamic investigation and treatment. She had suffered from urinary incontinence since 1960. In 1961, 1962, 1967 and 1972 she was treated by means of vaginal repair operations resulting in only a few months of continence.

Careful history taking revealed that the urinary symptoms were mostly frequency, urgency, urge incontinence and nocturia 4 times. Medium fill water cystometry revealed detrusor hyperreflexia (Fig 1). Urodynamic examination with simultaneous pressure flow EMG recording during voiding was normal. Micturition-cysto-urethrography was normal and no objective stress incontinence could be demonstrated.

Treatment was started with parasympatholytic drugs with only minimal effect (Fig 2). Sacral nerve blockade was ineffective (Fig 3). Careful neurological examination revealed symptoms of cervical spinal cord compression but no peripheral paresis. Myelography showed prolapsed cervical discs (Cv vi, Cv vii) (Fig 4). The patient was treated with anterior cervical spondylodesis a.m. Cloward (5) in November 1975.

The urinary symptoms gradually decreased postoperatively and cystometry 18 months later was normal (Fig 5). At three year follow up (November 1978) she had neither frequency, urgency nor urinary incontinence, only nocturia at times. The patient was cured.

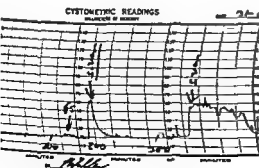


Fig 1 31.1.1975 Cystometry before treatment showing detrusor hyperreflexia (FS = first sensation, given = no loss).

## CYSTOMETRIC READINGS

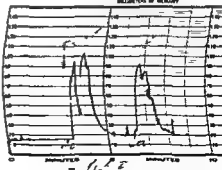


Fig 2 28.V.1975 Cystometry after 4 months treatment with parasympatholytic drugs (methantheline bromide (Banthine®)) 50 mg q.i.d. Detrusor hyperreflexia.

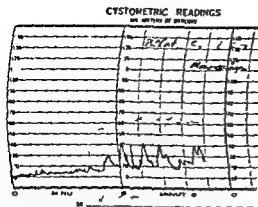


Fig 3 28 V 1975 Cystometry after bilateral blockade of  $S_{II}$  and  $S_{III}$  with bupivacaine (Marcaine®). Detrusor hyperreflexia

### CONCLUSION

Cystometry is the reflex hammer of the urologist and all patients referred with bladder disturbances to gynecologist, neurologist or urologist should be studied using cystometry. (1) If cystometry reveals signs of neurogenic bladder, supranuclear or infranuclear paresis. (6) a careful neurological history and examination must be undertaken. If this investigation indicates central or peripheral neurogenic lesions, further neuro-urological investigation must be performed with close collaboration between the neurologist and urologist. (2)



Fig 4 Cervical myelography showing prolapsed cervical intervertebral disc (C<sub>5</sub>-C<sub>6</sub>/C<sub>6</sub>-C<sub>7</sub>)

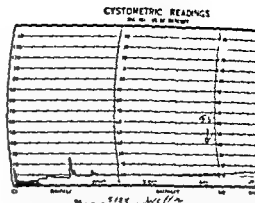


Fig 5 9 V 1977 Cystometry 18 months after a. tenor. dylosis vertebrae cervicalis a m. Cloward. Norz. tometry

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Submitted for publication January 25 1979

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## CASE REPORT

MÜLLERIAN MALFORMATIONS AND SIMULTANEOUS PREGNANCIES  
IN DIDELPHYS UTERI

## Review and report of a case

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7 Congenital anomalies originating in the müllerian are among the causes of gynecological and obstetrical anomalies that are commonly overlooked. These cases have a rate of 1.1-3.5 per cent in hystero-graphic examinations.

Authors have stressed the need to keep this type of anomaly in mind and to try to diagnose these conditions especially when the patients are examined for trivial reasons in the non pregnant state as well as during the prepartum or after delivery.

The case presented is a multiparous Beduin woman with simultaneous pregnancies in both cavities of an unexpected müllerian anomaly. The first twin female 2 000 gram was born at term and the second female 2 250 gram by cesarean section following unsuccessful attempts at vaginal delivery. The reasons for undertaking surgery was taken for common clinical reasons like inertia uteri and high presentation. An early diagnosis should result in adequate management and the earlier end of labor.

Didelphys in the living adult was first mentioned in 1873 when Frankel cited the cases of Oliver and Smith as the only two recorded cases (1). Pfannenstiel was the first to make a definite contribution in this direction when in 1894 he tabulated 12 cases of müllerian didelphys in adults. Miller (1) presented 5 cases (34 of the literature and one of his own). Only 3 cases presenting two distinct fundi each with its own cervix were considered when collecting these cases. Stolper found 10 in 7 400 married women. Bauer found 3 cases in 19 000 examinations (2). (3) established 1 case in 1 458-7 040 pregnancies but 1 in 27 703-28 389 women and only one of twins. Berkeley *et al* (Tortora) (4) consider a twin pregnancy as slightly more than seven times commoner in the double uterus than in the nor-

## CASE REPORT

A 30-year-old Beduin woman para 6 two newborns with low birthweight died after delivery. At the time she was delivered at home of a female fetus birthweight being 2 000 gram and the placenta was expelled a few minutes after admission to hospital. Abdominal examination revealed a second live fetus high head presentation mild uterine contractions cervical dilatation of 1.5 cm. X ray blood and urine tests revealed no abnormality. Amniotomy was performed but no progress was noted. The next day a pitocin drip was attempted and 20 hours after the first delivery a cesarean section was performed. Surprisingly two uteri were found the fetus being in the left one. The size of the left uterus was adequate to a term pregnancy and the right uterus of a size adequate for a four months pregnancy. Each uterus had one tube and one round ligament on the external surface. The peritoneal fold between the two uteri was easily visible. A segmental transverse incision in the left uterus was made and a female normal fetus was born birthweight being 2 250 gram. The placenta was normal. Four months after delivery a hystero-graphy was performed and two uteri with two cervices were seen. Intravenous urography revealed no abnormalities.

## COMMENT

The term didelphys is derived from two greek words meaning double uterus. More accurately the condition consists of two distinct and complete halves rather than a true reduplication of a normal uterus so that each uterus has only one set of ligaments and appendages arising from each lateral border. Usually the term double uterus is applied to any condition resulting from the failure of complete fusion of the müllerian ducts (5).

The incidence found by different authors varies from 1/321 to 1/625 deliveries. We found 29 anomalies in 23 085 deliveries including 4 cases of double uterus or 1/796.





Fig 1 Didelphys uteri after cesarean section in the left side

Different classifications have been suggested by different authors. Classified according to müllerian origin Semmens (6) presents two groups

*Group I* functional uteri of single müllerian origin  
Bicornis bicollis unicornis bicornis unicollis

*Group II* functional uteri of dual müllerian origin  
Bicornis uterus subseptus arcuatus

Uterus didelphys is most likely to be found first during adolescence when the patient complains of some menstrual disturbance secondly at the time of premarital examination thirdly after marriage when the patient complains of dyspareunia or unexplained abortion and last during pregnancy

Hematometra is commonly associated with a double uterus (2) as are abortions. Unusual fertility is demonstrated in many cases. Fetal wastage is estimated between 22 per cent and 33 per cent (2, 6). There is a great liability to premature labor (3) and labor is prolonged as a result of poor muscular development of the uterus a small rigid cervix and the encroaching non pregnant uterus (2). The incidence of breech presentations rises to 20 per cent in malformed uterus in transverse lie to 52 per cent in retained placenta to 15 per cent. Maternal mortality is comparatively high and there is a higher rate of fetal and newborn mortality (3).

The duplicity of the uterus presents numerous instances of what may well be assumed to be examples of superfetation. A case of twin pregnancy is reported by Dubierre where the interval between deliveries was 14 weeks. Lustner finds a maximum interval of 74 days [both cited by (2)].

Physical signs observed at routine prenatal examinations that should arouse suspicion of congenital uterine anomalies are fetal limb flanking with over

lie breech flanking with contralateral limb flanking fetal limb flanking with fundal notching cervical position in the uterus positive Piscachek's sign pregnancy and axial deviation of the uterus. One has stressed notching and broadening of the fundus a floating head at term in a primipara the absence of accepted causes of disproportion as well as abnormal lie recurring breech presentation trapped or retained placenta and a prolonged stage of labor. All these constitute indications for post partum hystero-graphic evaluation which confirms the anomaly in almost 100 per cent of the cases accordingly to Hoy [cited by Semmens (6)].

If manual removal of the placenta becomes necessary obstetricians should be aware of the phenomenon of triangular spasm and cornual polyp which are accentuated by the use of oxytocin in non pregnant women. Probing of the uterus with a uterine sound will often confirm the presence of a septum (8). During dilatation and curettage it is obligatory to assess the continuity of the uterus from one horn to the other with a blunt instrument so that the presence of any complete or partial septum may be noted. Hystero-graphically congenitally malformed uteri show rather rounded and club-like horns whereas normal uteri have relatively straight horns (8). In the 36 cases of Semmens (6) intravenous pyelograms were done and 25 per cent showed agenesis of one kidney and 8 per cent kidney anomalies. A possible genetic factor in the etiology of uterine malformations has been postulated by Polshuk (9).

Fig 2 Hystero-graphy four months after the last

The management of labor in cases with uterine anomalies is controversial. Findley (2) writes that treatment should not differ at all from that in the constituted uterus. Hoffman (7) prefers an elective cesarean section. Inertia uteri should be managed by abdominal delivery rather than oxytocin stimulating effect of which would cause horn rupture, abnormal fundal contraction patterns and fetal separation (6). A vaginal septum should be removed as soon as it is recognized, preferably in the gravid state (6).

Although in our case it cannot be proved conclusively that the first twin was delivered of the right uterus, signs like the size of the right uterus that have been seen during the intervention, the unusual expulsion of the first placenta before the delivery of the second twin, and the physiological cervical opening of the left uterus, allow us to assume that both pregnancies were simultaneous in the right and left uteri.

The case was managed without a previous diagnosis of double uterus. Surgical intervention is indicated in obstetrical common reasons like inertia uteri and malpresentation. However, the thought of the possibility of a uterine malformation even in a multiparous woman should surely prevent a delay in diagnosis and adequate management.

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*Submitted for publication September 21, 1978*

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## CASE REPORT

EARLY DIAGNOSTIC SIGNS IN THE DEVELOPMENT OF  
A FALLOPIAN TUBE CARCINOMA

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**Abstract** A woman is presented in whom cellular atypia signs of abnormal fibrinolysis in a jet washing caused instant suspicion of malignancy after a benign curettage. Nearly 3 years later a fallopian tube carcinoma was diagnosed. The value of determination of biological markers of plasma in addition to the cytologic interpretation of the fluid is emphasized.

Carcinoma of the fallopian tubes is extremely rare. Its incidence is reported to be 0.38 per cent of all gynecologic malignancies. In most cases the true diagnosis is unknown prior to operation and the disease is usually found in an advanced stage (1-3). In 1958-1969 the incidence in Malmö (adult female area 280 000 inhabitants) was 1.2 per cent, i.e. 7 out of 595 gynecological malignancies (2).

Some cases of malignant ovarian tumors (Fibrinolytic Degradation Products FDP (8)) are frequently found in high concentrations in ascitic fluid. Thus in theory malignant endometrial tumors could accordingly be accompanied by increased levels in uterine fluid. In 1975 when testing the Jet Washer (4) we therefore began to determine FDP in Jet Wash samples in addition to the cytological interpretations (5).

This is a case report of a patient in whom high concentrations of FDP in uterine fluid caused suspicion of malignancy nearly 3 years before a fallopian tube carcinoma was diagnosed.

## CASE HISTORY

A 50-year-old woman with menopause at 50 years of age had a routine gynecologic health check up in 1975. The clinical examination was normal, but the vaginal smear revealed atypia of glandular cells. Because of this a Gravlee Jet Washer and a curettage were performed. The cytologic diagnosis was atypia without signs of malignancy, but further

control was recommended. The FDP were found to be abnormally increased. The histologic diagnosis after curettage was postclimacteric endometrium without sign of atypia.

The presence of FDP, particularly in combination with the cytological atypia, caused suspicion of malignancy and the patient underwent hysterosalpingography, hysteroscopy and another curettage. All these examinations were normal. Gynecologic examination and further vaginal smears during the next 2 years were completely normal.

In the beginning of 1978 the patient complained of urinary incontinence and a watery discharge was seen in the vagina. She was referred for a complete urogynecologic investigation which did not reveal any abnormalities. At a gynecologic examination under anesthesia, however, a tumor with a diameter of about 3 cm was palpated at the site of the right adnexa. Curettage revealed postclimacteric endometrium.

Ultrasonographic examination confirmed the existence of an adnexal tumor and cytologic examination of the vaginal discharge revealed atypical glandular cells with the same morphology as described in 1975. Another hysterosalpingography was performed and showed a tumor at the right side of the uterus with a probable connection to the right fallopian tube.

Laparotomy was performed and revealed an enlarged right fallopian tube containing clear serous fluid. The distal end of the tube was occluded by a 2.5 cm in diameter, hard, grey-white tumor which was adherent to the right pelvic wall. The ovaries as well as the uterus were of normal size and appearance. Both the adnexa were removed together with the uterus. The histopathological diagnosis was adenocarcinoma of the right fallopian tube.

## DISCUSSION

Most probably the fallopian tube carcinoma was already present in 1975, nearly 3 years before the tumor was clinically diagnosed.

Despite extensive examinations the only pathological findings were the presence of cell atypia without suspicion of malignancy in a routine vaginal smear and in an endometrial Jet Wash. High concentrations of FDP were also noted in the Jet Wash sample. The significance of the latter was at that time uncertain.

The results of all other conventional examinations were normal. The cytologic atypia in the Jet Wash was interpreted as a probably false positive finding (6) and subsequent vaginal smears were normal.

In the beginning of 1978 the patient complained of urinary incontinence which she first noted in a mild form as early as in 1976. The symptoms of carcinoma of the fallopian tubes are vague and in most cases so slight that the disease is highly advanced before the patient seeks care. However, one of the symptoms may be an abnormal watery discharge interpreted as urinary incontinence.

Since 1975 we have found abnormally elevated ( $\geq 10$  mg/l) concentrations of FDP in uterine fluid in 71 per cent of patients with endometrial malignancies (12 out of 17) and in 38 per cent of patients with ovarian malignancies (6 out of 16).

The value of the FDP test is limited because of a relatively large number of false positive results (approximately 10 per cent) and has been replaced by lactate dehydrogenase (LD) isoenzyme analyses in the diagnosis of endometrial malignancies (7).

A new chemical method for the analysis of a tumor activator of fibrinolysis has however recently been developed (11, 12). Later tests have shown elevated concentrations of this tumor activator and of FDP as well as an abnormal LD-isoenzyme pattern in the uterine fluid collected from the present patient prior to operation.

Therefore, in view of this somewhat unusual case it seems justified to suggest that the addition of biochemical analysis to the cytological interpretation of uterine fluid could be of value in the early diagnosis of gynecologic malignancies.

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Submitted for publication February 14, 1979

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## CASE REPORT

## PRIMARY CARCINOMA OF THE BARTHOLIN GLAND

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A report of a patient with a Bartholin gland tumor is presented. Although this disease is rare it should be in mind when treating common Bartholin gland tumors. Bartholin gland carcinomas are misdiagnosed as common cysts or abscesses in half of the cases leading to a considerable delay in diagnosis. A review of the literature concerning this disease and recommendations are made.

Primary malignant tumors of the Bartholin gland are rare. Klob reported the first case in 1864 and 200 cases have subsequently been reported in the world literature. The clinical appearance of these tumors is very similar to that of a common Bartholin gland cyst which is illustrated by the following case report.

## CASE REPORT

The patient is a 32 year old para 4 who consulted the out-patient clinic of the hospital after observation of a firm and tender mass in the region of the left Bartholin gland. The tumor had been present for 3 months but had increased in size appreciably during this period. The only symptom she had experienced was some degree of dyspareunia. There was no history of previous inflammation of the Bartholin gland. At examination there was a 2 x 2 cm firm moveable nontender nodule in the area of the Bartholin gland on the left side. Overlying skin was intact. The rest of the vulva was normal as was the gynecological examination finding otherwise. No pathological changes were found in the inguinal regions. The finding was diagnosed as a left sided Bartholin cyst. Excision of the cyst was performed 5 weeks later. The tumor was adherent to the underlying muscle but could be removed without difficulty. The specimen measured 1.5 x 2 cm and was firm without cystic cavity. On macroscopic examination the tumor was seen to arise from a moderate hematoma the postoperative wound was normal. Histological examination showed a low differentiated carcinoma of the adeno-acanthoma type completely not radically removed.

Radical vulvectomy with skin transplantation (but not lymphadenectomy) was carried out three weeks after the first operation. Histological examination showed no remnants of cancer. Control examinations over a 9 month period have not shown any signs of recurrence and the patient has had no complaints.

## DISCUSSION

This case fulfils the four criteria for diagnosis of a primary cancer of the Bartholin gland as established by Honan (1897):

- 1 correct anatomic position of the tumor
- 2 location of the tumor deep in the labium
- 3 overlying skin intact
- 4 presence of some elements of glandular epithelium

These criteria are very strict and have probably decreased the apparent incidence of cancer originating from the Bartholin gland (1) although this type of tumor accounts for 3-8 per cent of all vulvar cancers (2).

Because of a different clinical behavior cancer of the Bartholin gland should be distinguished from other vulvar carcinomas.

The median patient age for the diagnosis of Bartholin gland cancer is 53 years (3) which is nearly a decade earlier than that for common vulvar cancers.

Histologically about 40 per cent of the malignant tumors in the Bartholin gland are squamous cell carcinomas 46 per cent adenocarcinomas 8 per cent adenoid cystic carcinomas and 6 per cent carcinomas melanomas or undifferentiated malignant tumors (1, 4, 5). Adenoacanthoma as reported here is in the literature generally described as being a mixture of squamous cell carcinoma and adenocarcinoma.

The most common symptom reported by other authors is the subjective observation of a tumor in the vulva (4) and this was true in the present case. The tumor may be painful and cause dyspareunia. On ex-

amination there is an enlargement of the Bartholin gland often hard and indurated but there may be a cystic degeneration. In approximately half of the cases reported in the literature the initial clinical diagnosis is that of a benign cyst or inflammatory process. This results in a delay of 3 months to several years in establishing the correct diagnosis (3). It is important to remember that uncomplicated inflammatory disease of the Bartholin gland is uncommon after 40 years of age and does not occur at all in the postmenopausal years (6).

Chamlian (3) recommends biopsy in the following situations:

- 1 if induration is noted during incision or marsupialization of Bartholin gland enlargement
- 2 if necrotic material is drained
- 3 if there is slow healing after drainage or marsupialization

Lürman (7) suggests that all Bartholin gland enlargements should be subject to biopsy.

Because of the small number of patients reported in the literature no uniform mode of treatment has been accepted. However most authors recommend surgical treatment. The surgical procedure varies from local excision to extensive vulvectomy including dissection of the inguinal femoral external iliac common iliac hypogastric obturator lower aortic and vena caval nodes.

Chamlian (3) states that it is wise to tailor treatment to the individual patient rather than to advocate a standard mode of therapy for all patients.

Most authors recommend radical vulvectomy combined with a bilateral inguinal and femoral lymph node dissection (2, 4, 8). Trelford (2) emphasizes the importance of dissecting deeply down to the urogenital diaphragm. Lymphadenectomy is generally not necessary in adenoid cystic carcinoma of the Bartholin gland since this type of carcinoma is less aggressive and rarely exhibits regional or distant metastases (5).

## CONCLUSIONS

It is important to be aware of the existence of a malignant Bartholin gland tumor in case of enlargement of the Bartholin gland with characteristics as described above. All Bartholin gland cysts which are not cured should be examined histologically.

## ACKNOWLEDGEMENT

The authors wish to thank Professor N. Nilsson for reading and revising the manuscript.

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Submitted for publication November 28, 1979

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# SERUM LACTIC ACID DEHYDROGENASE AND ISOENZYMES DURING PREGNANCY AND LABOR

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**Abstract** Total serum lactic acid dehydrogenase activity (LDH) and the levels of LDH isoenzymes were investigated in 4 women during early pregnancy (8-16th week) in 28 women during late pregnancy (29-37th week) in 73 at term (38-42nd week) and in 27 during labor (38-42nd week). LDH activity was found to be elevated in severe pre-eclampsia and in chronic hypertensive women during pregnancy as well as during normal and dysfunctional labor. No change was established in total serum LDH during normal pregnancy.

LDH 1 was increased during late pregnancy and at term. In severe pre-eclampsia and during normal labor it was decreased. LDH 2 was also decreased in severe pre-eclampsia and during dysfunctional labor. LDH 3 was decreased during late pregnancy but increased in severe pre-eclampsia. No change was observed in LDH 4 during pregnancy or in labor. LDH 5 was increased in normal and dysfunctional labor.

Lactic acid dehydrogenase (LDH) is an enzyme found in all tissues working the anaerobic metabolism of carbohydrates catalyzing pyruvate to lactate. Lactic acid dehydrogenase is especially abundant in skeletal muscle where its activity is greater than in smooth muscle such as in the uterine muscle (22, 23, 37).

During normal pregnancy many enzymes are more active than in the non-pregnant state. Most reports on LDH activity during labor show an increase (1, 3, 8, 11, 13, 15, 17, 18, 21, 26, 27, 28, 38, 39, 40).

There are only a few investigations concerning enzymes in sera during dysfunctional labor. Only Briliantova *et al* (3) have studied LDH. They found a decreased serum LDH activity during dysfunctional labor.

The lactic dehydrogenase present in blood and tissues is separable into five isoenzymes by electrophoresis (LDH 1, LDH 2, LDH 3, LDH 4 and LDH 5). The relative proportions of the individual isoenzymes vary from tissue to tissue. The pattern of change in the normal serum isoenzyme pattern characterizes the tissue source of increased serum enzyme activity. Lactic dehydrogenase is normally present inside the cell but is passed into the serum after destruction of the cell or due to increased permeability of the cell.

This study was undertaken to establish the effect of pregnancy and labor, especially dysfunctional labor, on serum lactic dehydrogenase and LDH isoenzyme levels. The effects of pre-eclampsia and chronic hypertension were also investigated.

Table 1 The patients investigated

Types of patients	No. of patients	Week of pregnancy		Age (years)		Parity		
		Mean	SD	Range	Mean	SD	Mean	Range
1st pregnant	16				42.6	7.3	1.9	0-6
2nd pregnancy	14	11.0	1.5	8-16	30.6	8.5	3.6	1-7
3rd pregnancy	22	33.3	3.1	29-37	26.6	6.3	1.6	1-3
with severe pre eclampsia	6	33.2	2.6		28.0	4.1	1.3	1-2
at term	60	40.2	1.1	38-42	28.4	5.8	1.7	1-5
with mild pre-eclampsia	5	39.4	1.1		24.8	7.1	1.2	1-2
with hypertension	8	39.3	1.0		30.4	7.1	2.1	1-3
normal labor	13	40.2	1.2		26.6	7.3	1.6	1-4
primary dysfunctional labor	10	40.4	1.1		27.3	7.5	1.2	1-3
secondary dysfunctional labor	4	39.5	1.9		23.5	1.9	1.0	0



Table II Total LDH (I U) and LDH isoenzymes (per cent) in sera of non pregnant and pregnant women

	LDH		LDH 1		LDH 2		LDH 3		LDH 4		LDH 5
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	
Non pregnant (16)	262.4	18.9	25.6	0.1	38.7	0.7	25.1	1.1	4.9	0.7	51.6
Early pregnancy (8-16 wk) (14)	210.9	14.3	28.5	1.6	38.1	0.7	23.8	1.4	4.4	0.5	52.6
Late pregnancy (17-37 wk) (21)	284.3	7.7	31.1	1.0	39.9	0.6	20.7	0.8	3.7	0.3	46.6
with severe pre-eclampsia (6)	460.5 <sup>4</sup>	50.2	26.5 <sup>3</sup>	1.6	35.2 <sup>3</sup>	1.7	24.6 <sup>3</sup>	1.3	4.3	1.5	9.9 <sup>1</sup>
At term (38-42 wk) (60)	301.3	9.0	28.3	0.6	37.8	0.5	22.9	0.6	4.8	0.3	6.1
with mild pre-eclampsia (3)	263.0	19.1	27.6	2.0	35.9	1.4	24.3	1.9	4.9	1.2	7.3
with hypertension (1-8)	355.8 <sup>5</sup>	12.6	25.1	1.5	35.9	1.9	24.7	1.6	5.9 <sup>6</sup>	0.7	9.1

1  $p < 0.05$  from non pregnant 3  $p < 0.05$  from late pregnancy 5  $p < 0.001$  from term  
 2  $p < 0.001$  from non pregnant 4  $p < 0.01$  from late pregnancy

## MATERIAL

The material consisted of 142 pregnant women 14 of whom were in early pregnancy (8-16 gestation weeks) 28 in late pregnancy (29-37 gestation weeks) 73 at term (38-42 gestation weeks) and 27 in labor. The classification of the material according to the course of pregnancy (normal/pre-eclampsia/hypertension) and labor (normal/primary dysfunctional/secondary dysfunctional) the week of pregnancy, age and parity is shown in Table I.

The pregnancies were divided into pre-eclampsia and hypertension by the criteria recommended by the American Committee of Maternal Welfare (6). 16 non pregnant women served as controls. They were healthy and were not using any hormonal contraception. The labor was regarded as normal when no disturbance was observed in the rate of cervical dilatation. When cervical dilatation was slow from the start of labor, the labor was classified primary dysfunctional. In secondary dysfunctional labor the delay of cervical dilatation appeared after a normal initiation of labor. All the blood samples in the labor groups were taken during the dilatation phase of labor.

## METHODS

The determinations of serum lactic dehydrogenase and LDH isoenzyme levels were made from clotted venous blood. Total lactic dehydrogenase (EC 1.1.1.27) activity

was determined according to the Scandinavian method (5) at +37°C using an OLLI 3 000 microchemical analyzer (Ollituite Oy, Ävenlän, Finland). Reagents made by AB Kabi (Sweden). Lactate dehydrogenase activity is expressed as units (I U) per litre of serum.

The fractionation of LDH isoenzymes was performed on cellulose acetate membrane (Beckman, California, U.S.A.). Electrophoresis was carried out at 150 V for 40 min after which the fractions were developed according to the method of Dade (Iso-Form LDH, Merz & Dade AG, Switzerland) on the membrane and scanned by a Beckman Model 110 densitometer (California, U.S.A.) to determine the percentage activity of each fraction.

The statistical analyses were made with Student's  $t$ -test and  $p < 0.05$  was regarded as statistically significant.

## RESULTS

The results shown in Table II indicate that in early pregnancy serum LDH 1 was increased ( $p < 0.05$ ) and LDH 3 decreased ( $p < 0.05$ ) compared with the non pregnant state. At term serum LDH 1 was significantly ( $p < 0.001$ ) increased. No change was observed in total serum LDH activity during pregnancy either early or at the end of pregnancy.

Table III Total LDH (I U) and LDH isoenzymes (per cent) in sera of women with normal and dysfunctional labor

	LDH		LDH 1		LDH 2		LDH 3		LDH 4		LDH 5
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	
At term (60)	301.3	9.0	28.3	0.6	37.8	0.5	22.9	0.6	4.8	0.3	6.1
Normal labor (11)	405.3	27.1	24.8	1.0	35.7	1.3	24.2	1.3	5.4	0.5	9.6
Primary dysfunctional labor (10)	365.6	11.7	26.4	1.6	34.9	0.9	24.2	1.2	4.3	0.8	9.1
Secondary dysfunctional labor (4)	649.0 <sup>1</sup>	475.4	27.2 <sup>4</sup>	0.5	33.1 <sup>5</sup>	0.5	23.5	1.9	5.9	1.1	1.1 <sup>6</sup>

1  $p < 0.05$  from term 2  $p < 0.01$  from term 3  $p < 0.001$  from term 4  $p < 0.05$  from normal labor 5  $p < 0.01$  from normal labor  
 6  $p < 0.01$  from primary dysfunctional labor

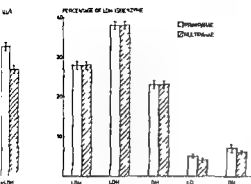


Fig. 1. Total LDH and isoenzymes in sera of primiparae and multiparae at term.

In patients with severe pre-eclampsia at 29-37 weeks gestation total serum LDH was higher ( $p < 0.01$ ) than in healthy pregnant women at that period of pregnancy (Table II). The same applied to LDH isoenzyme ( $p < 0.05$ ). The levels of LDH 1 and LDH 2 were decreased ( $p < 0.05$ ) in severe pre-eclampsia. In mild pre-eclampsia no changes were observed compared with the healthy pregnant women at the same gestation period (week 38-42) but in hyper-tensive women total serum LDH was higher ( $p < 0.001$ ) than in healthy women during pregnancy (Table II).

Total serum lactic dehydrogenase and LDH isoenzyme levels were investigated in 31 primiparae and in 19 multiparae at 38-42 weeks gestation. Fig. 1 shows that total serum LDH was greater ( $p < 0.001$ ) in primiparae ( $329.9 \pm 12.2$  IU/l) than in multiparae ( $201.1 \pm 10.1$  IU/l). No differences were detected in LDH isoenzyme levels.

During normal labor serum total LDH was very significantly ( $p < 0.001$ ) increased as it was during secondary dysfunctional labor ( $p < 0.001$ ) and significantly so ( $p < 0.01$ ) in primary dysfunctional labor compared with the same gestation period (week 38-42) without labor (Table III).

Table III shows that the LDH isoenzyme pattern was similar in primary and secondary dysfunctional labor. In contrast to the women at term LDH 2 was significantly ( $p < 0.01$ ) decreased in primary dysfunctional labor and very significantly ( $p < 0.001$ ) in secondary dysfunctional labor. LDH 5 was increased in primary ( $p < 0.05$ ) and secondary ( $p < 0.01$ ) dysfunctional labor. During normal labor LDH 1 was decreased ( $p < 0.01$ ) and LDH 5 increased ( $p < 0.01$ ) (Table III).

When the parturient groups were combined serum total LDH activity ( $p < 0.01$ ) and LDH 1 ( $p < 0.05$ ) increased more in secondary dysfunctional labor than in normal labor (Table III). In secondary dysfunctional labor the total LDH activity was augmented more ( $p < 0.01$ ) than in primary dysfunctional labor.

## DISCUSSION

The present investigation indicates that serum total LDH activity does not change during an uncomplicated pregnancy. This is in accordance with some authors (2, 4, 13, 16, 18, 19, 20, 21, 24, 29, 35, 36, 38, 40) while others report an increase of LDH activity in serum during pregnancy (7, 11, 14, 27, 32).

Electrophoresis differentiates the LDH isoenzymes by their speed of movement. LDH 1 being the fastest and LDH 5 the slowest. Tissues with high aerobic metabolism (cardiac muscle, brain, kidney and erythrocytes) are rich in the rapid type LDH isoenzymes 1 and 2 (H type). Skeletal muscle and liver have a preponderance of the slow type LDH isoenzymes 4 and 5 (M type) (31).

In the pregnant and non pregnant state the serum mostly contains the rapid kind of LDH isoenzymes, the muscle type (M type) being present in only small amounts (12, 41). Investigations into changes in the different LDH isoenzymes in serum during pregnancy do not agree. Hawkins & Whyley (12) and Miotti *et al.* (29) report an increase of LDH 1 during pregnancy in keeping with this study. LDH 3 and LDH 4 were found to be increased by Meade & Rosalke (27, 28) and LDH 4 by Pulkkinen & Willman (32) but a decrease of LDH 3 is reported by Miotti *et al.* (29). LDH 3 was decreased during pregnancy in this study too. The explanation for the increase of LDH 1 and the decrease of LDH 3 in late pregnancy in this study is not clear.

In pre-eclampsia Kubli (19) and Sammour *et al.* (34) found an increase in total serum LDH activity but no change is reported by some authors (4, 12, 20). In severe pre-eclampsia serum LDH activity was increased also in this study but neither total LDH nor LDH isoenzymes were changed in mild pre-eclampsia. LDH 1 and LDH 2 were decreased in severe pre-eclampsia as shown by Sammour *et al.* (34).

A probable source of release of LDH into the circulation in severe pre-eclampsia is the uterine muscle as LDH activity increases in the myometrium during pregnancy (10, 22, 25, 33, 37). The increased LDH 3 in severe pre-eclampsia is likely to originate from the

myometrium because Meade & Rosalki (28) show that LDH 3 and LDH 4 and Hawkins & Whyley (12) that LDH 2, LDH 3 and LDH 4 are the main LDH isoenzymes in the pregnant myometrium. Placenta is suggested to be the origin of the increased serum LDH in pre eclampsia as LDH 4 and LDH 5 were increased in serum and in the placenta in severe pre eclampsia (34). LDH 4 and LDH 5 were not changed in pre eclampsia in this study.

In chronic hypertensive women at term total LDH activity was higher than in healthy pregnant women as shown by Pulkkinen & Willman (32). Serum LDH activity was greater in primiparous than in multiparous women. This disagrees with the study of Pulkkinen & Willman (32) who claim more serum LDH in women during the third or subsequent pregnancy than in women in their first pregnancy.

The most prominent feature of the present study was a relatively marked increase in total LDH activity during labor. Such an increase has been presented in many other studies (1, 3, 8, 11, 13, 15, 17, 18, 21, 26, 27, 28, 31, 39, 40).

Placenta is regarded as the origin of increased serum lactic dehydrogenase activity during labor (8, 11, 21, 28, 38) as is muscle work (17, 26). The source of LDH during normal labor could be the uterine muscle as suggested by some authors (13, 15, 28, 39) because LDH activity increases in the myometrium during pregnancy (10, 22, 25, 33, 37).

The increase of total serum LDH was greatest in secondary dysfunctional labor. It presumably originates mainly from the uterine muscle as LDH activity in the myometrium is decreased in dysfunctional labor of this kind (25).

The muscle type (M type) LDH isoenzymes are known to be increased in serum during normal labor (12, 39). The isoenzyme LDH 5, a muscle type LDH isoenzyme, was increased during normal and dysfunctional labor in this study. As muscle type LDH isoenzymes are more active than the heart type (H type) in the myometrium during pregnancy (9, 10, 33) it is possible that the former are released from the myometrium into serum during labor. Skeletal muscle could also be the tissue from which LDH partly originates during labor as suggested by Unbehauen *et al.* (39). Another possible source of the elevated LDH 5 during labor could be the amniotic fluid cells since these tend to show increasing LDH 5 as pregnancy progresses (30) and an increase of LDH 5 in amniotic fluid with increasing gestational age has been confirmed (29). LDH isoenzyme 2 was decreased in dys-

functional labor in this investigation as well as with the study of Brilliantova *et al.* (3) who found a decrease of heart type (H type) LDH isoenzyme in dysfunctional labor.

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Submitted for publication July 27 1978

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# SPONTANEOUS LABOR AND ELECTIVE INDUCTION—A PROSPECTIVE RANDOMIZED STUDY

## II Bilirubin levels in the neonatal period

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**Abstract** The effect of induction of labor on the neonatal bilirubin levels was investigated in a prospective randomized study. Forty three infants born after elective induction with oxytocin using the Cardiff infusion system and 38 infants born after spontaneous delivery were studied. Several factors other than oxytocin administration which could influence the bilirubin levels such as gestational age placental transfusion and feeding routines did not differ between the groups. There were no differences with regard to pH in the umbilical vein Apgar scores haemoglobin and erythrocyte counts or bilirubin levels neonatally. Thus there was no evidence of an association between induction of labor and oxytocin and jaundice during the neonatal period.

Several earlier studies have shown an increased frequency of jaundice in the neonatal period after induced labor with oxytocin (5 7 9 12). Other authors have not found this increase (2 3 10 11 14). Most of these studies have been retrospective and factors such as intrauterine asphyxia gestational age dose of oxytocin and the placental transfusion have as a rule not been evaluated. Some authors have claimed that placental transfusion resulting from the induction and not oxytocin is the factor responsible for the increased bilirubin levels (4 6).

To clarify the relationship between induction of delivery with oxytocin and the development of hyperbilirubinemia remained unclear we decided to compare bilirubin levels in newborn infants in a random prospective study in spontaneous and induced labor at full term.

## MATERIAL

In this study the methods of selection and the methods of induction have been described in a previous paper (17). Only four infants 43 born after induced labor and 41 born after spontaneous labor were originally included. Three of the infants in the spontaneous group were born after prolonged pregnancies and were induced. They were therefore excluded from this study.

## METHODS

Immediately after the delivery the infants were placed on the delivery bed at the level of the vulva and were left there until the cord had been clamped. A blood sample was drawn from the umbilical vein exactly 60 seconds after delivery and the cord was clamped. After that 0.20 mg Methergine<sup>®</sup> was given intravenously to the mother. The oxytocin infusion was stopped and the dose given recorded. The cord blood was analyzed for pH haemoglobin (Hb) and hematocrit (Hct).

The infants were followed with daily capillary bilirubin measurements until decreasing levels were obtained. All infants were followed at least until day three and a maximal value was noted for all of them. At 74-72 hours of age venous hemoglobin and hematocrit were estimated. There were no cases of immunization involving the Rh or ABO systems. All infants were delivered at full term. A maturity assessment using external characteristics (8) was performed on day two on all infants. A routine physical examination was performed on day one and on day five to six. All infants were breast fed on demand. Naked weight was recorded daily.

The investigation was approved by the Ethical Committee of the University of Linköping.

## RESULTS

There were no differences in bilirubin levels Hb and Hct between infants of primiparae and multiparae or between the two obstetrical departments. Therefore the results were pooled. All infants were healthy at the two routine examinations. One infant in the induced group had a slight postnatal asphyxia. Seven infants developed cephal hematomas five of these in the spontaneous group. Birth weight lowest weight during the first week gestational age from the menstrual history gestational age according to maturity assessment Apgar scores and pH in the umbilical vein are presented in Table I. There were no statistically significant differences between the two groups for any of these parameters. There were no differences between the two groups with respect to hemoglobin and hematocrit in the umbilical vein hemo-

Table I Birth weight lowest weight during the first week gestational age gestational age according to maturity assessment Apgar scores and pH in umbilical vein in induced and spontaneous labor (Mean  $\pm$  SD)

	Induced labor	Spontaneous labor
Birth weight g	3 642 $\pm$ 442	3 744 $\pm$ 455
Lowest weight during the first week g	3 376 $\pm$ 436	3 448 $\pm$ 458
Gestational age days	280 $\pm$ 1	285 $\pm$ 4
Gestational age according to maturity assessment days	290 $\pm$ 12	281 $\pm$ 8
Apgar score 1 min	8.8 $\pm$ 0.7	9.0 $\pm$ 0.4
Apgar score 5 min	9.9 $\pm$ 0.4	9.9 $\pm$ 0.3
pH umbilical vein	7.35 $\pm$ 0.07	7.33 $\pm$ 0.07

(n = 43) \* (n = 38)

globin and hematocrit day two bilirubin levels day 1–3 or maximal bilirubin level during the neonatal week (Table II). There was no difference regarding the day for starting weight gain. In the induction group the mean oxytocin dose was 4.94 IU (0.5–11 IU). There was no relation between total oxytocin dose and maximal bilirubin level. Seventeen infants (39.5 per cent) in the induction group and 19 (50.0 per cent) in the spontaneous group had bilirubin levels above 170  $\mu$ mol/l. A statistical analysis of all the results showed no relationship between maximal bilirubin level and uterine activity measured in Monte video units, fetal heart rate decelerations, Apgar scores, pH in the umbilical vein, hemoglobin or hematocrit day two. There was however a tendency for heavy babies (birth weight above 4 000 g) to have lower maximal bilirubin ( $p < 0.05$ ). Further, if the delivery time (from a cervical dilatation of 4 cm to delivery) was short ( $< 150$  minutes) there was a tendency that the maximal bilirubin level was higher ( $p < 0.05$ ) see Table III.

Table II Hemoglobin (Hb) and hematocrit (Hct) in umbilical vein hemoglobin and hematocrit day 2 (Hb 2 Hct 2) bilirubin levels day 1–3 (Bil 1 Bil 2 Bil 3) and maximal bilirubin levels (Bil max) in induced and spontaneous labor (Mean  $\pm$  SD)

	Induced labor	Spontaneous labor*
Hb g/l	152 $\pm$ 14	155 $\pm$ 14
Hct %	45.4 $\pm$ 4.7	47.2 $\pm$ 5.4
Hb 2 g/l	186 $\pm$ 14	184 $\pm$ 7.1
Hct 2 %	54.2 $\pm$ 5.0	52.4 $\pm$ 7.7
Bil 1 $\mu$ mol/l	80 $\pm$ 3	88 $\pm$ 30
Bil 2 $\mu$ mol/l	120 $\pm$ 36	119 $\pm$ 43
Bil 3 $\mu$ mol/l	145 $\pm$ 46	156 $\pm$ 40
Bil max $\mu$ mol/l	156 $\pm$ 61	157 $\pm$ 70

(n = 43) \* (n = 38)

## DISCUSSION

A correlation between induction of labor and neonatal jaundice was suggested by V al (12). In most studies reporting an increased bilirubin level after induction the increase has been small and probably without clinical significance. In most studies have been prospective. Factors such as gestational age, obstetrical analgesia as well as feeding routines can influence the bilirubin level neonatally (5). In the present study there were no differences between the two groups with regard to these factors and no difference in bilirubin levels were demonstrated between the groups. An analysis of the total material showed however that birth weight related to maximal bilirubin level during the first week. Infants with a birth weight of more than 4 000 g had a tendency to lower bilirubin levels.

An additional factor which might influence the bilirubin levels in some earlier studies is uterine contractions following induction of labor, thereby a possible increased frequency of contractions. In the present study, in which the deliveries were performed by intrauterine pressure measurement and fetal heart rate recordings (FHR), we found no differences in FHR-changes, Apgar score or pH in the umbilical vein at delivery between the groups. There were therefore any significant differences in uterine activity or duration of labor (17). Even though an analysis of the whole group did not show a difference between maximal bilirubin levels and duration of labor, a short labor ( $< 150$  minutes) was related to higher bilirubin levels.

An increased frequency of neonatal jaundice after induction of labor might to some extent be explained by increased placental transfusion. Sallander et al (18) showed that the placental transfusion was increased in infants with high bilirubin levels at 72 hours of age among primiparas as well as among full term infants. Factors such as

Table III Maximal bilirubin level during the first 60 minutes in infants with birth weight below or above 4000 g and infants born after long (above 150 minutes) or short (below 150 minutes) delivery in induced spontaneous labor (Mean)

	No of infants	Maximal bilirubin level
Birth weight		
< 4000 g	61	166 $\mu\text{mol/l}$
≥ 4000 g	20	132 $\mu\text{mol/l}$
Delivery time		
> 150 minutes	19	182 $\mu\text{mol/l}$
≤ 150 minutes	62	149 $\mu\text{mol/l}$

of clamping the umbilical cord, the time of giving oxytocic agents postnatally, or the position of the infants with regard to the uterus after delivery may influence the degree of placental transfusion and must be taken into consideration. In the present study these factors were kept constant. The time for clamping the cord, 60 seconds postnatally, was chosen because by then most of the placental transfusion has ceased (18). Oxytocic agents can increase placental transfusion and for this reason Methergin<sup>®</sup> was given immediately after clamping the cord. Because the position of the infant at delivery in relation to the uterus also influences the placental transfusion (18) the infants were placed on the delivered at the level of the vaginal outlet. At this level blood flow between placenta and infants is not influenced by gravity. Venous hemoglobin and hematocrit on days two and three (24–72 hours) were taken as simple indirect measurements of the size of the fetoplacental transfusion. Venous hematocrit is known to be related to the total red cell blood volume and thus the placental transfusion. Erythrocyte volume is fairly constant between 24–72 hours of age and no changes in postnatal hemoglobin or hematocrit suggesting that the placental transfusion must be the same in the two groups. No differences in clinical routines were found.

Thus, in the present study most factors which can influence the bilirubin levels neonatally were equal in the two groups. There were no differences in bilirubin levels on days 1 and 3 or in the maximal bilirubin levels during the neonatal period. Therefore the following conclusion can be reached: Induction of labor with oxytocin within the criteria of our study does not lead to increased neonatal bilirubin levels. It is not likely that oxytocin *per se* in-

creases the risk for neonatal jaundice. The half life of oxytocin in blood at parturition is short, about 4 minutes (13) and oxytocin is readily degraded by the kidneys and the liver (15). It seems therefore unlikely that the oxytocin given in significant amounts will enter the fetal circulation and interfere with the metabolism of bilirubin. In studies in which such a relation has been postulated, the increased bilirubin level is more likely to be the result of an increased frequency of preterm deliveries and postnatal asphyxia or an increased placental transfusion.

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*Submitted for publication November 1 1989*

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## SKIN BLOOD FLOW IN NORMAL PREGNANCY MEASURED BY VENOUS OCCLUSION PLETHYSMOGRAPHY OF THE HAND

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**Abstract** Total hand blood flow was measured by venous occlusion plethysmography in 14 healthy primigravida women. Consecutive measurements were made from the 15th of pregnancy until term and after delivery. During pregnancy the mean hand blood flow measured under resting conditions with the subjects supine increased steadily from 7.7 in early pregnancy to 28.9 ml/100 ml/min at term. Simultaneously the peripheral vascular resistance decreased from 19.3 to 3.3 PRU<sub>100</sub>. When examined at 6-16 weeks after delivery hand blood flow and peripheral resistance were not yet returned to normal.

Information about the peripheral circulation in pregnancy is sparse. Venous occlusion plethysmography is a convenient and reliable method for measuring blood flow in a limb segment and has been utilized in many studies on hand, forearm, foot and leg of pregnant women. With regard to forearm and leg blood flow the results are conflicting. Most authors have found an increase in the resting forearm blood flow during the course of pregnancy (7, 10, 15, 17) while others were unable to confirm any change at all (2, 8). Hand blood flow has been shown to be slightly increased (10), unchanged (2) or even decreased (14) as compared to non pregnant values. Foot blood flow was found to be moderately increased in late pregnancy.

Concerning hand blood flow there is general agreement that a considerable rise occurs in late pregnancy when compared with non pregnant values (2, 7, 8). However, the authors used different plethysmographic temperatures which obviously influenced the magnitude of blood flow increase. As the vascular tone of the skin is very sensitive to changes in temperature only a slight decrease in water temperature will cause a considerable vasoconstriction. In the non pregnant state changes in forearm blood flow reflect to a large extent changes in muscular blood flow (3, 9) and this was assumed also to be the case in pregnant women (15). Spetz and Jansson (17) made simultaneous measurements of total forearm

blood flow by plethysmography and of forearm muscular blood flow with the <sup>133</sup>Xe clearance technique in non pregnant and pregnant women and found a progressive increase in total forearm blood flow during normal pregnancy while muscular blood flow remained unchanged. In non pregnant subjects there was a good correlation between forearm blood flow measured by plethysmography and <sup>133</sup>Xe muscle clearance. These findings seem to indicate that the main increase in forearm blood flow during pregnancy is due to a rise in skin blood flow and not in muscle blood flow.

Changes in skin blood flow are obviously best studied by hand plethysmography since skin is the main part of the soft tissues of the hand. According to Abramson and Ferris (1) skin constitutes one third of the hand volume, muscle 16 per cent and bones and tendons the rest, the blood flow of which can be neglected. The present study was performed in order to investigate hand blood flow at resting conditions in a series of young healthy primigravidae using a constant plethysmographic water temperature of 34°C which has been shown to be the most reliable temperature at least in non pregnant subjects (5).

## MATERIAL AND METHODS

A group of 14 healthy primigravidae were selected for investigation. None had to be excluded because of complications of pregnancy. They were all outpatients at the antenatal clinic of the hospital. Their mean age was 25 years with a range from 20 to 30 years. The stage of pregnancy was calculated in complete weeks of gestation from the date of the last menstrual period. At the first examination which was performed at 18-16 weeks of pregnancy each woman was informed about the completely harmless investigation procedure and they all willingly cooperated in the study. Their deliveries subsequently took place between February and May 1976 at 38 to 41 weeks of pregnancy, the mean being 40 weeks. Five women were smokers and smoked between 5 and 20 cigarettes per day. None had smoked within an hour before the examination.

Table 1 Mean arterial pressure (MAP) heart rate (HR) hand blood flow (Q) and peripheral vascular resistance (PRU<sub>100</sub>) in 14 pregnant women at different stages of pregnancy Number of examinations (n)

Month of pregnancy	IV	V	VI	VII	VIII	IX	X	Post partum
MAP mm Hg	83±3	83±2	79±3	84±2	87±1	89±2	89±2	80±2
HR beats/min	74±2	76±4	77±2	79±1	82±4	84±1	86±2	67±2
Q ml/100 ml/min	7.7±2.0	8.2±2.1	13.8±2.4	20.6±2.5	24.1±4.6	24.5±2.1	28.9±2.3	11.0±1.1
PRU <sub>100</sub>	19.3±4.9	15.3±2.7	7.1±1.2	4.6±0.5	4.6±1.2	4.0±0.3	3.3±0.3	8.7±1.1
n	9	10	9	10	6	14	15	14

The experimental conditions were carefully standardised. All measurements were performed in the same laboratory with a room temperature of 22°C. Before starting the investigation all the women rested comfortably in bed for about 30 minutes. During the measurements the women were in the supine position but in late pregnancy they were slightly tilted to the side to avoid inferior vena cava hypotensive syndrome. Arterial blood pressure was determined in the left arm using a conventional sphygmomanometer.

Venous occlusion plethysmography was applied to the right hand which was enclosed in a loosely fitting rubber glove provided with a rubber diaphragm tightly applied to a metal plethysmograph. The plethysmograph was filled with water maintained at 34°C (5) by a thermostat. Hand blood flow was measured by recording the increase in hand volume caused by the arterial inflow during a given period of time when the venous out flow was obstructed by a 6 cm wide sphygmomanometer cuff placed at the wrist. The occluding pressure was at least 10 mm Hg below the arterial diastolic pressure, i.e. about 40 to 60 mm Hg in most instances. On each occasion about ten consecutive measurements were made with intervals of one minute. The mean of these measurements was calculated and taken as hand blood flow expressed in ml per 100 ml tissue per minute. The pulse rate was calculated from the plethysmographic recordings. The peripheral vascular resistance was calculated by dividing the mean arterial pressure (diastolic blood pressure plus one third of the pulse pressure) by the hand blood flow value and expressed in arbitrary peripheral resistance units (PRU<sub>100</sub>). Altogether 87 examinations were made during pregnancy and post partum within a period from 6–16 weeks after delivery. Each woman was examined on at least five separate occasions during the course of pregnancy.

Mean±standard error of the mean (M±SEM) of blood pressure, heart rate, hand blood flow and PRU<sub>100</sub> were calculated at different stages of pregnancy. The results were statistically compared using a pairing design *t* test. The difference was considered to be statistically significant when *p* was less than 0.02.

## RESULTS

Mean length of pregnancy at the time of delivery was 40±0 weeks. Mean infant birth weight was 3536±114 grams. All were single births. The results are summarised in Table 1. The mean arterial blood

remained unchanged during the time period pregnancy studied even if a non significant rise from 83±3 mm Hg at 15–16 weeks to 89±2 mm Hg at 37–40 weeks could be noticed. The post partum value 80±2 mm Hg was significantly lower than of the last 8 weeks of pregnancy. The heart rate increased from 74±2 at 15–16 weeks to 86±2 beats per minute at 37–40 weeks. This difference was statistically significant. Post partum the heart rate was significantly lower compared to late but not compared to early pregnancy. The main purpose of heart rate recordings was to make sure that the subjects were in an acceptable resting state during examinations.

Hand blood flow increased continuously during the entire pregnancy taking into account a between individual women (Fig. 1). The rise is obvious from Fig. 2 where the mean graphically plotted against time. There is a significant increase from 7.7±2.0 at 15–16 weeks to 28.9±2.3 at 37–40 weeks. Mean hand blood flow post partum 11.0±1.2 ml/100 ml/min was significantly lower than in late pregnancy but still higher than at 15 weeks of pregnancy. Hand blood flow of the women who were smokers did not differ from the non smokers. Their blood flow values were distributed within the values of the whole group.

As expected peripheral vascular resistance followed the changes in hand blood flow and was significantly lower in late than in early pregnancy. Post partum value 8.7±1.1 PRU<sub>100</sub> was significantly higher than that in late pregnancy but still lower than at 15–16 weeks. Hence blood flow and vascular resistance were not completely normal by 6 weeks after delivery. No correlation between birth weight and the magnitude of hand blood flow could be found.

Hand blood flow  
ml/min 100 ml

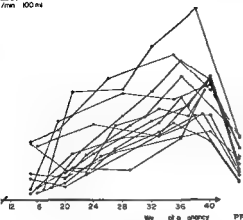


Fig. 1 Individual hand blood flow values of 14 women in relation to length of gestation and at 6-16 weeks after delivery (P P).

## DISCUSSION

The present study confirms the results of earlier investigations (2-8). Ginsburg and Duncan (8) found an increase in hand blood flow from 2.7 ml/100 ml/min before 14 weeks to 18.1 ml near term. The present comparable figures are 7.7 ml at 15-16 weeks and 28.9 ml/100 ml/min at 37-40 weeks of pregnancy. The difference between the present results and earlier findings may well be explained by the fact that Ginsburg and Duncan (8) used a plethysmographic water temperature of 32°C and a room temperature of 20°C while in the present study the corresponding temperatures were 34°C and 22°C respectively. Among other technical details the temperature factor is of significant importance when measuring such a sensitive parameter as hand blood flow. On the whole, individual values must be assessed with caution since the variability of the circulatory parameters is considerable between different series and between subjects within the series. The conclusion that can be drawn from Ginsburg and Duncan (8) and the present series, both studied under strictly standardised resting conditions, is that the mean increase in hand blood flow can be estimated to be about 20 ml/100 ml/min from early to late pregnancy. In the individual women there was an increase ranging from 11.5 to 49.5 ml/100 ml/min as shown in the present study. Earlier observations (11) indicating that the mean arterial pressure remains virtually unchanged during pregnancy were also confirmed. The pulse pressure

seems to be a little higher during pregnancy mainly because of a decreased diastolic pressure. The earlier reported increase in heart rate of about 15 beats per minute (11) was also observed in the present study. These changes start early in pregnancy as does the early rise in cardiac output (11).

The increased hand blood flow reflects mainly an increased skin blood flow. This indicates a progressive vasodilatation in the skin during pregnancy which is an old observation. Pregnant women feel warm, are most comfortable in a cold environment and their skin temperatures are high (6-10). The increase in blood flow is most pronounced in the distal parts of the extremities because skin constitutes the principal part of hands and feet whereas in the forearm skeletal muscle constitutes the greater part. Previous studies from this department (15-17) showed however that the mean forearm blood flow in late pregnancy was 12.3 ml/100 ml/min at a plethysmographic temperature of 36°C, 9.0 ml at 34°C and 6.9 ml at 26°C. This is still a considerable rise over the non-pregnant mean of 2.5 ml/100 ml/min at 34°C. The blood flow increase within the skin compartment of the legs is probably very small if any (2, 8, 10, 14). It has not been possible to measure skin blood flow in other regions of the body with presently available methods.

Ashton (4) found recently that smokers in the last trimester of pregnancy had higher foot blood flow and toe and finger temperature than non-smokers. He suggested that the rise in skin blood flow believed to occur in pregnancy applied mainly to smokers. This surprising suggestion is not supported by the present study where hand blood flow was equally high in smokers and non-smokers in the second half of pregnancy.

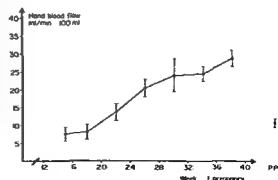


Fig. 2 Mean hand blood flow ( $M \pm SEM$ ) at different stages of pregnancy and at 6-16 weeks after delivery (P P).

The reason for the vasodilatation in the skin is obscure. Estrogens have been demonstrated to produce peripheral vasodilatation in sheep (18) but no changes in hand or forearm blood flow were observed when naturally occurring estrogens were given by infusion into the brachial artery of non pregnant subjects (13). On the other hand the vascular bed in pregnant women may react differently to hormonal stimuli compared to that in non pregnant women. The fact that hand blood flow was still elevated in the post partum period in the present as well as in Ginsburg and Duncan's (8) study does not support the concept that placental hormones are responsible for the vasodilatation in the vascular bed of the skin. A more probable explanation is the presence of decreased tone of the vascular smooth muscle due to a decreased sympathetic activity and/or to a decreased sensitivity to vasoconstrictor agents. No conclusions can be drawn from the present study with regard to structural changes of the vascular bed since for practical reasons it was not possible to achieve maximal vasodilatation.

The physiological purpose of an increased skin blood flow may be to dispose of the increased energy produced by maternal and fetal sources during pregnancy. From this point of view it would seem reasonable that skin blood flow should be greater if the fetus was large and thus produced more energy. There was however no correlation between fetal weight and hand blood flow.

Previous studies (12-16) have shown a reduced peripheral blood flow in pregnancies complicated by toxæmia and it has been suggested that this peripheral vasoconstriction takes place mainly in the skin (12). The relation between toxæmia and peripheral circulation is incompletely understood. The present study of skin blood flow in the hand is aimed to serve as a basis for further studies in pregnancies complicated by hypertension.

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Submitted for publication June 10 1978

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# SERUM LEVELS OF HUMAN PLACENTAL LACTOGEN DURING AND AFTER PRENATAL DEXAMETHASONE THERAPY

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**act** Thirteen pregnant women were treated with 4 mg dexamethasone intramuscularly three times daily for seven days during the last trimester of pregnancy in order to prevent the respiratory distress syndrome (RDS) in the neonate. The administration of human placental lactogen (HPL) was continued daily during the treatment and at intervals of 2-3 days afterwards. No changes in HPL were seen during the treatment. One week after the treatment had been stopped a statistically significant decrease in HPL was observed (p < 0.01). The levels returned to normal during the follow-up week. It is presumed that the observed decrease in HPL can be regarded as a result of a direct depression of placental function caused by the dexamethasone therapy. The clinical implications of this finding for the fetus are not known, but in cases of placental insufficiency glucocorticoid treatment should not be employed.

Physiologic treatment of pregnant women with symptoms of threatened premature birth using high doses of glucocorticoids has become increasingly common. After Liggins and Howie (12) demonstrated that this treatment is able to reduce the incidence of the respiratory distress syndrome (RDS) in premature infants. The dosage of glucocorticoid (betamethasone) employed by Liggins and Howie (12) was empiric. It is still not clear exactly what dosage of corticosteroid should be employed or for how long in order to obtain an optimal effect with regard to the prevention of RDS.

Very little is known of the risks to the unborn infant involved in the treatment, in particular possible long-term risks have recently been the subject of discussion (1, 7).

The treatment brings about a considerable suppression of both the serum levels of estradiol and the excretion of estradiol in the urine (3, 18). Thus this parameter could not be employed for evaluating the condition of the feto-placental unit as long as the treatment is in progress; neither may it be used in the period immediately following discontinuation of the therapy. It has been shown that no change takes place in the serum levels of human placental lactogen (HPL) during the treatment of pregnant women with beta

methasone (13) or with dexamethasone (26). For this reason it has been suggested that HPL levels are a reliable parameter during glucocorticoid treatment.

In the present investigation the serum levels of maternal HPL have been followed during and after treatment with dexamethasone in consecutive cases where birth did not occur in connection with the treatment.

## MATERIAL AND METHODS

During a 1 year period 32 patients were admitted to the Department of Obstetrics and Gynecology of the Hølding Hospital in the last trimester of pregnancy with symptoms of threatened premature birth. Indications for the commencement of treatment with 4 mg dexamethasone intramuscularly 3 times daily for a maximum of 7 days were present in all of these patients. Thirteen of them did not give birth in connection with the treatment and comprise the patient material.

Three of the pregnancies were twin pregnancies. Four of the patients had at that time been subjected to cerclage of the cervix. Apart from these factors the pregnancies were uncomplicated; in particular none of the patients suffered from hypertension or symptoms of toxemia of pregnancy. None of them were disposed to diabetes mellitus or had signs of intrauterine growth retardation.

All of the patients were kept in bed and all but one were in accordance with the regime of the department put on the following additional treatment: phenobarbital orally in doses not exceeding 100 mg daily and opium suppositories of 50 mg 3 times per day. Five of the patients were also given progesterone suppositories of 500 mg 3 times per day. Only one of the patients in this material received beta-mimetic drugs. The additional treatment was commenced at almost the same time as the dexamethasone treatment and was not changed during or in the week following the dexamethasone treatment.

On the day before and each morning of the treatment a blood sample was withdrawn for the determination of HPL levels. Blood samples were also withdrawn at intervals of 2-3 days during the two weeks following the discontinuation of the dexamethasone treatment.

Serum levels of HPL were determined by radioimmunoassay (The Radiochemical Centre HPL Immunoassay Kit<sup>®</sup> Amersham).

The two-way analysis of variance was employed in the statistical analyses of the results.

Table 1 Individual and mean HPL levels in 13 patients before during and one and two week, with dexamethasone 12 mg per day for 7 days

Patient	Weeks of gestation	Serum levels of HPL mg/l			
		Before	1 week	2 weeks	3 weeks
1	34	7.3	7.2	6.3	6.2
2	34	4.7	5.4	5.4	4.9
3	31	12.8	13.6	8.7	15.0
4	30	5.7	5.5	5.1	6.8
5	32	7.2	6.4	6.0	6.4
6	34	9.0	8.0	7.4	8.1
7	30	6.6	6.8	5.8	7.0
8	31	6.8	7.2	6.3	8.5
9	32	9.2	9.4	8.9	9.8
10	32	8.4	9.1	7.8	10.1
11	33	3.4	3.1	2.8	3.7
12	34	8.2	7.6	7.0	7.7
13	29	4.8	4.9	3.7	4.9
	Mean	7.24	7.25	6.25	7.62
	SD	2.42	2.57	1.78	2.90
	P		ns	<0.01	ns

twin pregnancy

## RESULTS

The results of the determination of the serum levels of HPL obtained before and during treatment with dexamethasone showed almost no variation and no significant changes were demonstrated. The values obtained immediately prior to and at the end of the dexamethasone treatment are shown in Table 1. One week after the discontinuation of treatment there was a statistically significant fall in the HPL levels ( $p < 0.01$ ) however after a further week these had risen to a level above the initial values but this increase was not statistically significant. The rise corresponded to that expected as a result of the increase in gestational age.

All the patients later gave birth to healthy infants no signs were present in either mothers or infants which could be interpreted as indicating side effects or complications to the dexamethasone therapy.

## DISCUSSION

The synthesis of HPL takes place in the syncytiotrophoblast layer of the placental epithelium (16) and several investigations have shown that there is a significant positive correlation between maternal serum levels of HPL and the weight of the placenta (10, 15, 17). According to Samaan *et al.* (14) and Spona and Janisch (21) the synthesis of HPL is

autonomous and it has been demonstrated synthesis is unaffected by factors such as posture (25) or circadian variation (6, 22). A number of studies suggest that metabolic changes in the mother affect the synthesis of HPL. Thus hypoglycemia brings about a rise in HPL levels, hyperglycemia causes a fall (5, 8, 19, 24) and diabetic women in the third trimester of pregnancy have been unable to bring about changes in HPL levels corresponding to blood glucose when the latter are brought under control by oral glucose tolerance tests (6, 9).

It appears to be well documented that measurements of HPL are a good indication of the functional condition of the placenta (10, 11) and would therefore be reasonable to presume that a fall in HPL levels is an expression of a reduced placental function and that this in turn would equal or increase the risk of fetal distress.

The levels of HPL were unchanged during treatment in the present investigation this is in agreement with earlier studies (13, 26). However a statistically significant fall in HPL levels was demonstrated during the week following the discontinuation of treatment but this was a transitory phenomenon as much as normal values were again observed one week later.

The treatment of non-pregnant women with corticoids causes a displacement of the metabo-

ards gluconeogenesis. According to Tuimala *et al* this effect appears to be less pronounced in pregnant women than in normal subjects during dexamethasone treatment. Thus they found no changes in blood glucose but a rise in serum free fatty acids and serum insulin levels. As these changes are closely related to the steroid treatment they cannot explain the changes in HPL levels found in the present investigation.

The fall in HPL levels also appears to be unrelated to changes in serum levels of estradiol as these occur during glucocorticoid treatment.

Dexamethasone treatment in the present study was given for a period of 7 days. Theoretically there is the possibility that treatment with dexamethasone for a long period would bring the patient into a state of relative suprarenal gland insufficiency with subsequent changes in the metabolic status. However, if this be correct it would be more reasonable to expect a compensatory rise in HPL levels rather than the decline demonstrated in the present investigation. Until this question has been studied further one must assume that dexamethasone treatment of our patients has had a direct inhibitory effect on placental HPL.

The slight fall in serum levels of HPL as demonstrated here will presumably be of no clinical importance in the majority of cases but in patients where fetal function is already affected dexamethasone treatment may cause a further reduction with a consequent risk to the fetus. This might be a possible explanation of the relatively higher mortality following dexamethasone treatment both antenatally and neonatally found by Liggins and Howe (12) in the complicated by symptoms of severe toxemia of pregnancy.

Despite the present treatment with dexamethasone because of the good results obtained by Caspi *et al*.

However, we consider it necessary to point out the possible danger of extending the treatment with high doses of glucocorticoid to so many days. Treatment should only be commenced on strict indications and where possible first after evaluation of the L/S ratio. Conditions that influence the fetal function should be considered to be contraindications to antepartum glucocorticoid therapy.

#### ACKNOWLEDGEMENT

This work was supported by the Danish Medical Research Council.

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*Submitted for publication August 24 1978*

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## FETAL SYSTOLIC TIME INTERVALS AFTER PARACERVICAL BLOCK DURING LABOR

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## METHODS

**Record.** Fetal systolic time intervals (FSTI) were recorded 10th second (s) during uterine contractions in 25 patients in the first stage of labor before and after paracervical block (PCB). Twenty-two patients had good or excellent relief of pain. The newborn infants were not depressed after PCB. The duration of the R-R and S<sub>1</sub>-S<sub>2</sub> intervals was measured. A close correlation was found between the R-R and S<sub>1</sub>-S<sub>2</sub> intervals both before and after PCB and during early decelerations recorded before and after PCB. The S<sub>1</sub>-S<sub>2</sub> interval was prolonged after PCB even after correction for the heart rate.

The R-R interval and the amplitude of the first part of the first heart sound (S<sub>1</sub>) also increased after PCB.

The FSTI showed a conspicuous cyclic variation during each contraction. The S<sub>1</sub> amplitude decreased after the start of contraction, the others increased simultaneously. These results contradict the assertion that PCB causes fetal hypoxia or reduction of the maternal placental circulation. Centralization of the fetal circulation, possibly combined with a direct effect of local anesthetic on the fetal heart, might explain the changes found after PCB.

The cyclic variation of the parameters during uterine contraction is probably caused by a transfer of blood between the placenta and fetus. The changes of myometrial pressure after PCB, as shown by the alteration of the shape of the amniotic pressure curve, are compatible with a transfer of blood.

The effects of local anesthetics on the mechanical function of the fetal heart have not been studied in vivo. Active systolic pressure and contractility (P/dt) of the left ventricle is decreased by mepivaine in the isolated human heart from midpregnancy and atrioventricular and intraventricular conduction is delayed. Decreased contractility prolongs the QT and the Q-S<sub>2</sub> interval in the adult human (8). The present investigation was undertaken to study the effect of PCB on fetal cardiac performance during labor.

PCB was given as described before (11, 12) using bupivacaine with adrenaline (Marcain-Adrenalin<sup>®</sup> Bofors) 40 mg-40 µg fluid volume 16 ml divided into 4 doses. Care was taken to place the local anesthetic solution as superficially as possible at points where the patient felt puncture pain which she could locate to the correct side of her body. Minimal axial force was applied to the Lofbak needle to avoid distortion of the lateral vaginal fornices. The supine hypotensive syndrome was avoided.

The fetal electrocardiogram (FECG) was recorded with a scalp electrode and the fetal phonocardiogram (FPCG) from the maternal abdomen as earlier described (13). The amniotic pressure was recorded with an open ended catheter (11) and the phases of the amniotic pressure curve were calculated as described previously (12). The peak pressure of uterine contractions was designated active pressure (AP). AP = total pressure minus resting pressure (11) and the time when AP occurred, time of active pressure (t<sub>AP</sub>). t<sub>AP</sub> was chosen as zero time. The parameters were read every 10 seconds (s) with the first reading point 40 s before t<sub>AP</sub> and the last 40 s after t<sub>AP</sub>. Deviations up to 3 s from a reading point were allowed to avoid FPCG disturbance. Each plot was the mean of 3-5 individual readings.

From the FECG curve the duration of the cardiac cycle (R-R interval), PR time and RS wave duration were measured. The fetal electrical axis was calculated (20) according to the principle of Larks (15). From the FPCG curve the duration of mechanical systole (S<sub>1</sub>-S<sub>2</sub> interval) and the first and second heart sounds (S<sub>1</sub> and S<sub>2</sub>) were measured. Correct timing of the start of S<sub>1</sub> was controlled by counting the number of deflections of S<sub>1</sub>. The amplitude of the two parts of S<sub>1</sub> recognized (S<sub>1a</sub> and S<sub>1b</sub>) and the two parts of S<sub>2</sub> (A<sub>2</sub> and P<sub>2</sub>) were measured to the nearest 0.5 mm.

The R-S<sub>1</sub> interval was measured as the time from the crossing of the first limb of the R wave with the FECG base line to the crossing of the first high frequency deflection of S<sub>1</sub> with the FPCG base line. The R-S<sub>1</sub> interval was used instead of the Q-S<sub>1</sub> interval due to variation of the Q wave. It represents the electromechanical lag period and a part of the isovolumetric ventricular contraction (8). Time intervals were measured to the nearest 2.5 millisecond (ms), the paper speed was 100 mm/s.

Patients. PCB was offered to patients who obviously suf-

Table I Clinical features of patients and their newborns

	No. of patients		Mean	Range
Nulliparae	18	Maternal age years	24.8	18-31
Multiparae	7	Duration of pregnancy days from LMP	284	274-293
Admitted for early labor	13	Friedman index after PCB cm/hour	5.1	0.8-12
ruptured membranes spontaneous start of labor within 24 hours	6	Weight of newborn infant g	3769	2960-4390
induction	6	Apgar score 1 min of age	8.4	4-9
Infusion of oxytocin for induction	6	Apgar score 5 min of age	9.1	4-10
for stimulation	3	Weight of placenta g	691	400-1100
Miscolored amniotic fluid before PCB	3	Length of umbilical cord cm	54.2	32-83

\*One newborn infant got Apgar 4 points at 1 and 5 min of age (occiput posterior position secondary arrest of labor). Newborn infants got Apgar 6 points at 1 min of age (Pethidine given to the mothers after PCB). The later course of all newborn infants complicated.

ferred from the pain of labor and had only received small doses of  $N_2O$  30 per cent in  $O_2$ . The FPCG recording should be distinct with minimal noise. Twenty-nine parturients with a single fetus without mechanical disproportion and with the fetal head engaged in the pelvis were included.

Four cases were excluded due to disturbance of the FPCG curve especially before PCB. The readable parts of the curves showed no significant deviations from the means of the remainders.

**Statistics.** By comparing all the values before PCB ( $n=225$ ) with all the values after PCB the standard error of the difference was calculated for each parameter. At each reading point ( $n=25$ ) the significance of the differences were evaluated by the Wilcoxon signed rank test for paired differences. The two-tailed test as some results were not normally distributed. Correlations were calculated on a desk computer and the statistical significance was calculated as described by Armitage (3).

## RESULTS

Table I shows clinical details. Twelve patients got complete relief of pain and were unable to report contractions. 5 of them fell asleep. Ten cases had a good effect but they could feel some discomfort or pressure. In 3 parturients the PCB was less effective another dose PCB or pethidine being necessary 45-60 minutes after the first. Seven patients had pronounced subjective and objective evidence of hyperventilation (paresthesia, numbness, presence of Chvostek's sign or carpal spasm) which disappeared a few minutes after PCB. Acid base studies were not performed. Maternal blood pressure did not change significantly during the experiment.

The PR time did not change during uterine contractions. The mean duration before PCB was 102.5

ms (range 85-120 ms) and after PCB 100.1 ms (range 85-120 ms). The QRS axis showed a deviation to the left at  $t_{AP}$  both before and after PCB. Between contractions the mean axis was  $+113^\circ$  (range  $113^\circ-177^\circ$ ) before PCB and  $+141^\circ$  (range  $120^\circ-174^\circ$ ) after it. The RS wave duration of the FPCG was not influenced by uterine contractions.

**R-R Interval.** The mean duration of the R-R interval before PCB ( $n=225$ ) was 442.4 ms (1 minute) and after PCB 457.2 ms (131 beats). The difference is statistically significant ( $P<0.0005$ ). Disregarding short time variation and changes greater than 20 ms between contractions, fetuses had an increased R-R interval after PCB.

Table II shows the mean values at each point. After PCB the R-R interval was significantly prolonged only after  $t_{AP}$  giving its mean a biphasic or cyclic course.

**$S_1-S_2$  Interval.** The  $S_1-S_2$  interval before PCB was 194.1 ms and after PCB 201.3 ms afterwards ( $P<0.0005$ ).

Comparing each reading point before and after PCB (Table II) the differences are significant except 10 and 20 s before  $t_{AP}$ .

The duration of the R-R and  $S_1-S_2$  intervals were closely correlated before PCB ( $r=0.65$ ,  $P<0.0005$ ) after PCB ( $r=0.65$ ,  $P<0.0005$ ). The regression of  $S_1-S_2$  on R-R before PCB ( $y=0.25x+83$ ) and after PCB ( $y=0.17x+124$ ) in the observation range testing the difference between the slopes (3) give  $P>0.3$ .

Correcting the  $S_1-S_2$  interval for heart

Table II Duration of the R-R, S<sub>1</sub>-S<sub>2</sub>, S<sub>1</sub>-S<sub>2c</sub> and R-S<sub>1</sub> intervals at each reading point before and after milliseconds means and standard error of the means SE

		Reading points (seconds)								
		-40	-30	-20	-10	t <sub>AP</sub>	+10	+20	+30	+40
PCB										
mean		442.9	438.2	444.1	443.2	447.7	445.7	442.0	434.5	443.4
SE		8.2	8.3	6.3	5.1	10.6	8.5	5.7	5.4	6.1
S <sub>2</sub>										
mean		192.0	191.2	195.6	195.3	195.7	195.7	193.1	193.3	195.0
SE		3.1	3.2	2.3	2.6	2.5	2.7	2.2	2.1	2.1
S <sub>2c</sub>										
mean		288.6	288.8	294.0	293.5	293.0	293.3	290.5	293.2	293.1
SE		3.2	3.0	2.3	3.5	2.4	2.3	2.4	2.3	2.6
S <sub>1</sub>										
mean		32.9	33.1	32.8	33.5	33.7	34.3	34.0	33.5	33.3
SE		1.1	1.0	1.1	1.2	1.1	1.1	1.3	1.1	1.1
PCB										
mean		449.9	443.6	436.5	452.9	471.9	469.6	463.3	466.4	460.7
SE		7.5	6.9	5.8	7.4	11.1	11.2	10.7	10.2	9.4
S <sub>1</sub>										
mean		200.4 <sup>b</sup>	197.3 <sup>a</sup>	196.8	198.9	201.9 <sup>b</sup>	202.9	203.9	206.0	203.6
SE		2.3	2.3	2.7	2.4	2.3	2.3	2.4	2.3	2.0
S <sub>2c</sub>										
mean		299.1 <sup>c</sup>	296.4 <sup>b</sup>	297.8	295.8	294.7	296.8 <sup>a</sup>	300.2 <sup>c</sup>	301.4	300.6
SE		2.5	2.5	3.0	2.4	2.9	3.0	2.5	3.0	2.8
S <sub>1</sub>										
mean		34.9	35.7	35.0 <sup>b</sup>	35.6	37.0	37.5 <sup>b</sup>	36.7	37.1	37.2
SE		1.1	1.1	1.2	1.6	1.7	1.7	1.6	2.0	1.8

At each reading point the values before and after PCB are compared using Wilcoxon's signed rank test for paired differences, no symbol  $p < 0.05$  <sup>b</sup> $p < 0.01$  <sup>c</sup> $p < 0.001$

$S_1 - S_2$  where the R-R interval is given in seconds (2)

$S_{2c}$  by the use of Bazett's formula (4)

$$S_1 - S_{2c} = \frac{S_1 - S_2}{\sqrt{R - R}} \quad (2)$$

mechanical systole was still longer after PCB than before (Table II). The S<sub>1</sub>-S<sub>2</sub> and S<sub>1</sub>-S<sub>2c</sub> intervals showed cyclic variation during uterine contrac-

case got early decelerations (9) starting 20 min after PCB and persisting until delivery (Fig 1). A close correlation between the R-R and S<sub>1</sub>-S<sub>2</sub> intervals  $r = 0.81$   $p < 0.001$ .

In another case had early decelerations both before and after PCB. Correlating the S<sub>1</sub>-S<sub>2</sub> interval with the R-R interval before PCB gave  $r = 0.89$   $p < 0.001$   $y = 0.15x + 123$   $n = 29$  and after PCB  $r = 0.90$   $p < 0.001$   $y = 0.15x + 125$   $n = 29$ .

In one case a typical PCB bradycardia developed 6 min after PCB lasting about 7 minutes. During this period of bradycardia the correlation between the R-R and S<sub>1</sub>-S<sub>2</sub> intervals persisted and the S<sub>1</sub>-S<sub>2c</sub> interval remained unchanged.

**S<sub>1</sub> interval.** Recorded between contractions before PCB the R-S<sub>1</sub> interval correlated with the heart rate of the newborn  $r = 0.66$   $p < 0.001$ . It was 8.5% longer after PCB than before.

Considering each reading point the R-S<sub>1</sub> interval also increased after t<sub>AP</sub> revealing a cyclic pattern during uterine contraction which was more pronounced after PCB (Table II). The increase did not correlate with AP. Using the reading point 40 s before t<sub>AP</sub> as a reference the increase was not statistically significant before PCB. After PCB the increase was statistically significant at 10 and 40 s after t<sub>AP</sub>.

There was no statistically significant correlation between the R-S<sub>1</sub> and R-S<sub>2</sub> intervals.

In the fetus with PCB bradycardia the R-S<sub>1</sub> interval varied between 32.5 and 35 ms before PCB and between 35 and 37.5 ms afterwards. In one case (Fig 2) variable decelerations (9) were registered during the second stage of labor and the R-S<sub>1</sub> interval increased from 30 ms between contractions to 37.5 ms during decelerations. The umbilical cord of the newborn was tightly encircled around the neck. One of the cases excluded due to FPCG noise before PCB had typical variable decelerations starting some 40 minutes after PCB the R-S<sub>1</sub> interval increased from 30 ms between contractions to 42.5 ms during decelerations.

**S<sub>1</sub> amplitude.** The S<sub>1</sub> amplitude was increased by a mean of 3.8 per cent after PCB. A cyclic pattern was seen during contractions with the lower values after

Table III Amplitude of the two parts of the first heart sound  $S_{1a}$  and  $S_{1b}$  at each reading point before and after PCB mm means and standard error of the means SE

		Reading points (seconds)								
		-40	-30	-20	-10	$t_{AP}$	+10	+20	+30	+40
<b>Before PCB</b>										
$S_{1a}$	mean	14.2	13.9	14.2	13.8	13.2	14.3	12.4	12.8	13.9
	SE	1.6	1.2	1.5	1.4	1.5	1.7	1.2	1.3	1.5
$S_{1b}$	mean	12.9	12.5	12.1	11.4	11.6	10.2	10.4	10.6	10.9
	SE	1.0	0.9	0.9	0.8	0.7	0.7	0.7	0.7	0.8
<b>After PCB</b>										
$S_{1a}$	mean	15.8 <sup>a</sup>	15.8	16.1	15.4	13.7	13.4	13.8	12.2	13.2
	SE	1.3	1.5	1.6	1.8	1.4	1.5	1.4	1.2	1.2
$S_{1b}$	mean	11.1	10.7	10.5	10.7	10.4	9.3	9.3	9.1	9.8
	SE	1.0	0.9	0.7	0.7	0.7	0.6	0.6	0.7	0.8

Statistical comparison see footnote in Table I

$t_{AP}$  (Table III) The decrease after  $t_{AP}$  was not statistically significant before PCB. After PCB the  $S_{1a}$  amplitude was significantly lower 30 and 40 s after  $t_{AP}$  than 20, 30 and 40 s before  $t_{AP}$ .

In the fetus with PCB bradycardia the heart rate was constant for a short period during maximal bradycardia with R-R interval 692.5–700 ms (87–86 beats/minute). The  $S_{1a}$  amplitude and the  $S_1$ – $S_2$  interval during this period showed a distinct variation. Measuring 14 consecutive heart cycles the correlation between these parameters was significant statistically  $r=0.88$ ,  $p<0.001$ ,  $y=0.37x+223$  ( $S_{1a}=x$ ).

$S_{1b}$  amplitude The mean values at each reading point were lower after PCB than before (Table III). Before PCB the means 10, 20 and 30 s after  $t_{AP}$  were significantly lower than 40 s before  $t_{AP}$ . The same statistically significant changes were found after PCB.

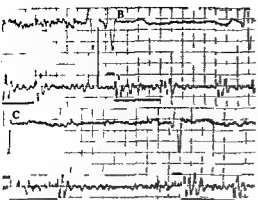
$S_2$  amplitude The amplitudes of  $A_2$  and  $P_2$  analyzed separately. After PCB the  $A_2$  amplitude showed a small, statistically insignificant fall. The amplitude did not change after PCB. No cyclical variation of the  $A_2$  or  $P_2$  amplitudes took place. Uterine contractions Twenty patients received a uterine infusion during the experiment to secure uterine activity (Table I). Before PCB AP from 27 to 99 mm Hg, after PCB from 30 to 97 mm Hg. AP did not change significantly after PCB (Table I) but the frequency of contractions decreased ( $p<0.01$ ). The duration of the phases of the pressure curve changed after PCB (Table I) found previously (12).

Forty s before  $t_{AP}$  the amniotic fluid pressure was not increased significantly in any patient. Then at this reading point therefore represents the fetal circulatory status in the fetus between contractions.

Table IV Uterine contractions before and after PCB duration of phases seconds, active pressure (AP) mm Hg, maximal rate of pressure increase (dP/dt max) mm Hg/s and frequency number of contractions per minutes means and standard error of the means SE

	$A_2$	$A_1$	$A_d$	$D_2$	$D_1$	$A+D_1$	AP	dP/dt max <sup>d</sup>	F
<b>Before PCB</b>									
mean	21.8	4.0	13.5	12.5	4.7	55.3	30.9	3.0	5.3
SE	1.7	0.8	1.2	1.0	1.1	2.0	4.0	0.2	0.2
<b>After PCB</b>									
mean	14.3	8.3 <sup>c</sup>	11.2 <sup>b</sup>	8.2	8.1	49.5 <sup>b</sup>	34.3	2.6 <sup>b</sup>	4.7 <sup>c</sup>
SE	0.9	0.7	0.7	0.6	0.9	1.6	4.3	0.1	0.2

$A_2$  acceleration phase,  $A_1$  rectilinear phase,  $A_d$  deceleration phase of the ascending limb (A) of the amniotic pressure curve,  $D_2$  active phase,  $D_1$  rectilinear phase of the descending limb (D) of the amniotic pressure curve (12). Statistical comparison see footnote to Table I. <sup>d</sup>before statistical comparison two cases were excluded because the difference between AP before and after PCB was more than 5 mm Hg (dP/dt is proportional to AP (12)).



Case 18 25 min after PCB fetal head on pelvic floor  
 vy lines between the FECG and FPCG curves show the  
 $S_1$  interval duration below FPCG curve heavy lines  
 te the  $S_1-S_2$  interval duration  
 20 s before  $t_{AP}$  R-S<sub>1</sub> interval 40 ms R-R interval  
 20 ms  $S_1-S_2$  interval 195 ms  
 20 s after  $t_{AP}$  R-S<sub>1</sub> 32.5 ms R-R 625 ms  $S_1-S_2$  210  
 ms after  $t_{AP}$  R-S<sub>1</sub> 35 ms in the first 40 ms in the se  
 cardiac cycle R-R 800 ms  $S_1-S_2$  232.5 ms Note  
 R-S<sub>1</sub> interval increase in second heart cycle in C due  
 to increased amplitude of the first deflection of  $S_1$ . The  
 interval is disclosed by counting the number of deflections  
 and controlling the duration of  $S_1$ .

## DISCUSSION

R-R interval increased after PCB but  
 significantly so only after  $t_{AP}$ . In patients where the  
 contractions were continued the R-R interval had re-  
 turned to the level before  $t_{AP}$  10-20 s after the last  
 contraction point. A corresponding fall of fetal heart rate  
 during uterine contraction after PCB was found in an-  
 other study (14) where the last reading was taken at  
 the end of contraction to disclose late decelerations.  
 At this time the fetal heart rate had almost reached  
 the level it had before contraction. Thus it seems that  
 PCB favors the development of early decelerations.  
 Several of the parameters measured showed an ob-  
 vious cyclic variation during uterine contraction. The  
 R-R interval was not entirely regular but generally speak-  
 ing the R-R and R-S<sub>1</sub> intervals and to some extent  
 the  $S_1-S_2$  interval increased after  $t_{AP}$ . The  $S_{1a}$ ,  $S_{1b}$   
 and  $A_2$  amplitudes decreased after  $t_{AP}$ . The cyclic  
 variations were greater after PCB than before. Organ  
 (17) found that PEP increased during uterine  
 contraction in a similar manner to the R-S<sub>1</sub> interval  
 in the present study.

The same cyclic variation of the  $S_{1a}$  amplitude and  
 R-S<sub>1</sub> interval during uterine contraction was found  
 in the ectopic beats of a fetus with supraventricular  
 extrasystoles (13). A transfer of blood between the  
 placenta and fetus and/or increased resistance of the  
 fetal placental circulation was believed to cause the  
 changes, the ectopic beats being more sensitive to the  
 hemodynamic changes than the sinus or postectopic  
 beats which did not show cyclic variations.

If PCB brings about a centralization of blood in  
 the fetus, the transfer of blood between the fetus and  
 placenta would be enhanced, explaining the increased  
 cyclic variations observed during uterine contraction  
 after PCB.

Acidosis prolongs PEP (16) and reduces contrac-  
 tility (1) thus prolonging mechanical systole. Acido-  
 sis after PCB could explain the prolonged R-S<sub>1</sub> and  
 S<sub>1</sub>-S<sub>2</sub> intervals. PCB with a small dose of local anes-  
 thetic (lidocaine 100 mg with or without adrenaline)  
 injected superficially gave a fall of fetal scalp blood  
 pH and increased  $pCO_2$  (10). Signs of metabolic  
 acidosis did not develop. The fetal acidosis probably  
 was secondary to a transitory acidosis in the mother.  
 It seems likely that maternal metabolic acidosis  
 developed before PCB is masked by a respiratory  
 alkalosis due to hyperventilation. The pain relief of  
 PCB suddenly removes hyperventilation, revealing  
 the metabolic acidosis.

Teramo in 2 studies (21, 22) injected larger doses of  
 local anesthetic (mepivacaine 200-400 mg or bupiva-  
 caine 50-100 mg) deeply into the paracervical tissue  
 and afterwards found increased base deficit together  
 with  $pCO_2$  increase and pH fall. This technique ob-  
 viously may induce a fetal metabolic acidosis. As  
 several mothers in the present investigation had sub-  
 jective symptoms and objective signs of a respiratory  
 alkalosis before PCB, acid-base changes such as those  
 reported by Jacobson and Gårdmark (10) most likely  
 occurred in the fetuses. Symonds (20) showed that fe-  
 tal acidosis is correlated with a shift of the QRS axis  
 to the left. As the present series did not show any  
 QRS axis change after PCB, acidosis of the fetuses  
 must have been slight.

Many authors have reported a shortening of PEP  
 during hypoxia in the fetus (17, 18, 23, 26). The  
 R-S<sub>1</sub> interval increased after PCB. Even if the  
 R-S<sub>1</sub> interval constitutes only a part of PEP, it is  
 unlikely that a shortening of PEP would have occur-  
 red simultaneously as those parameters largely  
 describe the same physiological processes. Murata *et al.*  
 (16) in their extensive *in vivo* studies of the fetal

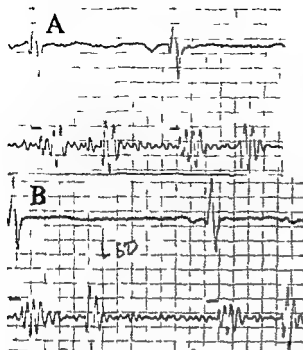


Fig 2 Case 20 The newborn infant had the umbilical cord tightly encircled around its neck. The heavy lines between the FECG and FPCG curves denote the duration of the R-S<sub>1</sub> interval

A 10 s before  $t_{AP}$  R-S<sub>1</sub> 30 ms R-R 475 ms S<sub>1</sub>-S<sub>2</sub> 215 ms

B 10 s after  $t_{AP}$  R-S<sub>1</sub> 37.5 ms R-R 680 ms S<sub>1</sub>-S<sub>2</sub> 247.5 ms

monkey found a small increase of PEP during nonacidemic hypoxia. However, in spite of a substantial  $pO_2$  fall, they found no direct correlation between PEP and  $pO_2$ .

The prolonged R-S<sub>1</sub> and S<sub>1</sub>-S<sub>2</sub> intervals after PCB might be caused by a direct effect of the local anesthetic on the fetal heart. Increased cardiac contractility, as evidenced by the increased S<sub>1a</sub> amplitude (19) and the unchanged relationship between the R-R and S<sub>1</sub>-S<sub>2</sub> intervals during early decelerations before and after PCB, discount this possibility. The variation of the S<sub>1a</sub> amplitude and S<sub>1</sub>-S<sub>2</sub> interval during PCB bradycardia with a constant R-R interval also speaks in favor of undisturbed myocardial contractility. The ventricles are heterogeneous with regard to muscle mass, fibre orientation, oxygen tension and catecholamine distribution (6). Variation of those factors might cause the beat to beat variability of fetal cardiac inotropism which was found. A toxic effect of local anesthetic would probably have muted this variation.

After late clamping of the umbilical cord a pro-

longation of PEP (25) and the S<sub>1</sub>-S<sub>2</sub> interval has been found. Accordingly, a centralization of the blood volume would also prolong the S<sub>1</sub>-S<sub>2</sub> interval, probably the R-S<sub>1</sub> intervals. A combined direct and indirect influence of the local anesthetic on the fetal circulation could be the best explanation of the changes found.

Yao *et al.* (24) have shown that the blood volume transferred from the placenta to the fetus, although the circulatory dynamics before and after PCB cannot be directly compared, their results suggest that blood can most probably be transferred from the placenta and the fetus during uterine contractions when the filling of the intervillous space is maximal. The periods of filling of the intervillous space (Fig 3) when the veins draining the intervillous space are closed and the arteries filling it are open, occur during the periods of uterine contractions. PCB has lowered the rate of amniotic pressure change. In this case, the filling periods after PCB would be prolonged, increasing the impact on the fetal circulation. But the moments of arterial and venous closure or opening are unknown; the duration nor the time of the filling periods are not determined.

In vitro studies of arteries from the hu-

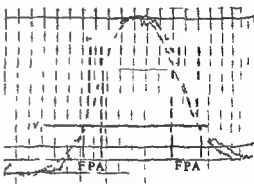


Fig 3 Case 8 Amniotic pressure curve of before PCB (dotted curve) superimposed on a curve of equal maximal amniotic pressure after PCB (solid curve)

V: arbitrary level of amniotic pressure at which the draining of the intervillous space closes on the descending limb of the amniotic pressure curve. A: the corresponding pressure for the arterial closure of the intervillous space. FPA: the filling periods of the intervillous space before PCB. FPA: the filling periods after PCB.

Even if the pressure gradient between V and A temporal localization varies, FPA will be longer than V or A do not change to any great extent after PCB.

(5) and *in vivo* experiments in ewes at term arterial injection of local anesthetic (7) have that local anesthetics due to arteriospasm decrease both placental and nonplacental uterine flow. As the cyclic variations of the fetal systolic time intervals found is believed to be caused by contraction of the intervillous space volume during uterine contraction, arteriospasm probably did not occur in the present series. Decreased arterial flow would decrease the intervillous space filling velocity and increase the cyclic variations after PCB. The whole of the hemodynamic changes after anaesthesia as judged by the method applied in the present series are small, excluding any substantial depression of fetal cardiac function. Higher doses of local anesthetic (21-22) are not necessary to achieve a sufficient analgesic effect and should be avoided. With the technique and dosage of anaesthetic applied, PCB is considered to be a safe method of pain relief during the first stage of labour in normal parturients.

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Submitted for publication September 6 1979

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# A COMPARISON OF THE THREE METHODS FOR EXTERNAL FETAL CARDIOGRAPHY

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Phonocardiography, abdominal electrocardiography and ultrasound cardiography are the three methods for external fetal cardiography. In the present study the methods have been compared regarding the quality of the records. The patients (163) were between the 34th and the 40th week of gestation. Graphs with less than 15 per cent of artifacts were found in 23.4 per cent for phonocardiography, 55.2 per cent for abdominal electrocardiography and 88.9 per cent for ultrasound cardiography. It is shown that the number of successful abdominal electrocardiograms was higher with advancing gestational age. The quality of the phonocardiograms was influenced by an anterior placenta. No obvious influence of a placental wall on external cardiography could be demonstrated.

Phonocardiography is one of the methods of surveillance in the antenatal period. Contrary to the situation during delivery, one is entirely dependent on external methods for fetal heart rate monitoring, i.e. phonocardiography, ultrasound cardiography and abdominal electrocardiography. At the beginning of this century efforts have been made to develop a phonocardiograph (21). The general use of phonocardiography became routine during the 1960s (7). Ultrasound cardiography was developed some years later (3, 14). The first commercially available equipment for abdominal electrocardiography was introduced in 1974 (2, 4, 6, 8, 20). External cardiographs of today integrate all three methods for external cardiography in the same unit. The clinical use and practical application of each external method have been investigated in many studies (5, 9, 10, 12, 13, 16, 17, 19) but only in a few has a comparison of the three methods at the same occasion and on the same patient been performed (1, 10). The aim of this study was to compare the three different external methods at the same time on the same patient, with special reference to the influence of the placental site and obesity.

## MATERIAL AND METHODS

During 18 months 163 antepartum recordings were obtained from 137 patients. One hundred and fifteen patients were monitored once and 22 two or three times with at least one week's interval. All patients were hospitalized and the recordings were obtained between 34 and 40 weeks of pregnancy. The patients were monitored between 1 and 5 p.m. and the woman adopted a lateral tilt of some 15° with a pillow under the right side of her back. Usually the bladder was emptied before the onset of registration.

The length and weight of the patients were registered. Obesity was defined as more than normal mean weight + 20 per cent (15). The position of the fetus, the maximal recording of the fetal heart sounds, and the best place for the cardiography transducers were marked on a chart. In 143 cases the placental site was determined ultrasonically.

A Hewlett Packard cardiotocograph model 8030 A was used equipped with heart sound transducer 1513 B, ultrasound transducer 1577 A (broad beam transducer) and an indifferent thigh electrode 15270 A. For abdominal cardiography disposable silver electrodes were used. The caudal electrode was placed in the midline above the symphysis. The cranial electrode was placed over the uterine fundus where the best fetal contact was found. The three methods of indirect cardiography were applied for 30 min each. After the initial application of the transducers (or electrodes) no further adjustment was made. The paper speed was 1 cm/min. All records were obtained and examined by the author. The percentage of artifacts or failures were expressed as follows:

$$\frac{\text{cm failure}}{\text{cm registration}} \times 100$$

The quality of the records was classified according to Ruitgers (17):

- < 5 % failure = very good quality
- > 5 < 15 % failure = good quality
- > 15 < 50 % failure = poor quality
- > 50 % failure = uninterpretable

## RESULTS

An acceptable graph (very good or good quality) was obtained in 92.6 per cent of the 163 cases with either one or more of the methods. In only 23 cases (14 per cent) however did all three methods succeed on the same occasion. Phonocardiography gave very good

Table I *Quality of phonocardiograms from 163 cases between 34 and 40 weeks of pregnancy*

Quality	per cent	no
<5% failure	2.5	4
>5<15% failure	20.9	34
>15<50% failure	36.8	60
>50% failure	39.8	65

or good records in 23.4 per cent (Table I). The corresponding figures for abdominal electrocardiography and ultrasound cardiography was 55.2 and 85.9 per cent respectively (Table II and III). In only one case was phonocardiography the only working method, compared with 9 for abdominal electrocardiography and 52 for ultrasound.

For phonocardiography the number of acceptable graphs never exceeded 32 per cent regardless of gestation. From the 37th week abdominal cardiography succeeded in about 70 per cent. Ultrasound gave good results regardless of the gestation (Fig. 1).

The placenta was localized by ultrasound scanning in 143 cases. Seventy-seven cases had anterior and 66 posterior placenta. The quality of the tracings obtained by phonocardiography was significantly lower with anterior compared with posterior placentas (Fig. 2). No such difference was found for abdominal electrocardiography and ultrasound cardiograms (Fig. 2). Among the studied patients 38 were defined as obese. The quality of the records from phono and abdominal cardiography seemed to be low in these obese groups (Fig. 3) but there is no statistical difference. Obviously obesity has no influence on the ultrasound cardiography.

In abdominal electrocardiography the fundal electrode was placed on the same side as the fetal abdomen in 58 per cent of the cases, in the middle in 24 per cent and on the side of the fetal back in 18 per cent of the cases.

Table II *Quality of abdominal electrocardiograms from 163 cases between 34 and 40 weeks of pregnancy*

Quality	per cent	no
<5% failure	24.5	40
>5<15% failure	30.7	50
>15<50% failure	6.8	11
>50% failure	38.0	62

Table III *Quality of ultrasound cardiograms 163 cases between 34 and 40 weeks of*

Quality	per cent	no
<5% failure	42.3	69
>5<15% failure	43.6	71
>15<50% failure	11.0	18
>50% failure	3.1	5

## DISCUSSION

The study was restricted to pregnant women between the 34th and the 40th week of pregnancy. The investigation was to study all three methods for cardiography. It has been shown previously that the abdominal electrocardiography will give results during the 28th to 34th week (4, 5, 8, 9, 10).

Under optimal conditions external measurements can be compared on the same patient at the same time. This can be done only for two methods simultaneously (13). It is impossible to place all three electrodes at the same time, and consequently three methods have to be used after one another was done in this study.

The reported results of phonocardiography are widely between 22 and 77 per cent tracings of acceptable quality (10, 16, 19). This may in part be due to different methods of evaluation. The same mode of interpretation as Rüttgers (17) was used. 10 per cent of the graphs in this study were for

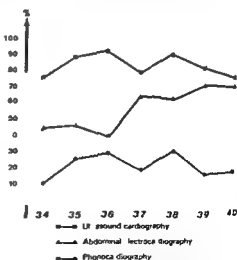


Fig. 1 Comparison of the three methods of cardiography related to gestation. The abscissa shows the week of pregnancy and the ordinate the percentage of graphs with less than 15 per cent failure.

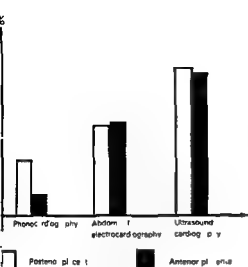


Fig 2 Comparison of cardiograms with <15 per cent failure from 66 cases of posterior placenta and 77 cases of anterior placenta

very good or good quality. Phonocardiography therefore gives a higher number of poor or uninterpretable graphs than the other two methods. The difficulties with this method are also illustrated by the fact that in only one case when ultrasound and abdominal electrocardiography failed did phonocardiography give an acceptable recording. From these results is the need for phonocardiography as standard equipment on cardiotocographs can be questioned. With ultrasound cardiography very good or good recordings were obtained in 85.8 per cent. The Doppler signal however is rather undefined and of considerable variation. (1) The equipment used in this study calculates the heart rate based on the mean frequency of three beats; consequently beat to beat variability is not calculated. Abdominal electrocardiography will produce signals defined enough to calculate beat to beat variability. (11) therefore in the interpretation of the variability this method gives a more reliable result than ultrasound cardiography. In the latter weeks of pregnancy especially the number of very good and good tracings is high and abdominal electrocardiography should be complementary to ultrasound. The placental site has some influence on the signals from external cardiotocography. (16-18) In agreement with other reports (5-9) this study showed no obvious influence from the placental site on the quality of abdominal electro- and ultrasound cardiography but there was a high number of poor and uninterpretable

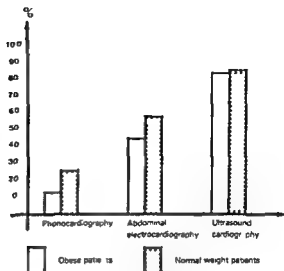


Fig 3 Comparison of cardiograms with <15 per cent failure from 38 patients with obesity and 125 patients with normal body weight

phonocardiograms when the placenta was situated anteriorly.

The influence of a thick abdominal wall on external cardiology is not quite clear. (5-18) In this investigation no obvious influence could be demonstrated.

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*Submitted for publication January 27, 1979*

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# ABRUPTIO PLACENTAE — TREATMENT WITH THE FIBRINOLYTIC INHIBITOR TRANEXAMIC ACID

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**Abstract** Abruption placentae is known to have a bad prognosis for the fetus. Pathologic proteolysis e.g. a pathologic activation of the coagulation mechanism and/or the fibrinolytic system is known to be a common complication in such cases. Analysis of the coagulation factors and components of the fibrinolytic system in the acute stage of 14 cases confirmed the earlier finding of mainly an activation of the fibrinolytic system which argues for the use of a specific fibrinolytic inhibitor. 73 consecutive cases of abruption placentae were treated with tranexamic acid in the acute stage. 67 of the patients were immediately delivered by cesarean section. The remaining six patients were in early gestational age and were treated for a prolonged period. The fetal mortality of the entire group was only 8 per cent, the maternal mortality nil. None of the cases were complicated by hemorrhagic diathesis or thromboses. We believe that routine immediate treatment with tranexamic acid reduces the perinatal mortality in cases of abruption placentae.

Abruption placentae is known to have a bad prognosis for the fetus. Perinatal death rates of 33 to 37 per cent have been reported most of the fetuses being dead on admission (11-20). Also maternal deaths (21-22) have been reported. Pathological proteolysis is a pathological activation of the coagulation mechanism and/or the fibrinolytic system is known to be a common complication (21). Recent studies have produced evidence that activation of the fibrinolytic system is often the predominant factor (6). This prompted us to try to reduce the complications associated with abruption placentae by treatment with the fibrinolytic inhibitor tranexamic acid. This report describes a consecutive series of 73 cases treated with tranexamic acid in the acute stage before delivery or for a prolonged period in women in an early stage of pregnancy.

## MATERIAL AND METHODS

383 deliveries. 73 were complicated by abruption placentae. These 73 women were given 1 g of tranexamic acid intravenously just before delivery by cesarean section. Six of the patients were in an early stage of pregnancy with less pronounced symptoms. In

these oral treatment with tranexamic acid in a dose of 1 g  $\times$  4 a day was continued until delivery (Table III) which also was performed by cesarean section. The diagnosis of abruption placentae was confirmed at examination of the placenta after delivery. Blood samples for determination of coagulation factors and components of the fibrinolytic system were obtained from 14 of the patients.

**Laboratory methods.** Normal values refer to 40 normal women in the 3rd trimester.

**Activated Partial Thromboplastin Time (APTT).** Automated APTT (General Diagnostics). Normal value <40 sec. **Platelet count** according to Björkman (3). Normal value 130 000–362 000/mm<sup>3</sup>.

**Fibrinogen.** The blood was collected with epsilon aminocaproic acid (EACA) and the fibrinogen was measured with the method of Nilsson and Olow (18). Normal value 4  $\pm$  0.7 g/l.

**Plasma P complex** (prothrombin + factor VII + factor X) was measured with the method of Owren and Aas (19). Normal range 124–200 per cent.

**Factor V levels** were measured with the method of Wolf (24). Normal value 104  $\pm$  12 per cent.

**Factor VIII activity** (VIII C) was estimated according to Nilsson *et al.* (16). Normal value 213  $\pm$  10.6 per cent. **Factor VIII antigen** (VIII R Ag) was determined according to Holmberg and Nilsson (12). Normal value 269  $\pm$  20.2 per cent.

**Fibrinolytic activity** of resuspended euglobulin precipitate on unheated fibrin plates (17). Normal value 0–65 mm<sup>2</sup>.

**Fibrin/fibrinogen degradation products (FDP)** were measured with the immunochemical method of Nielehn (15). In the presence of EACA and thrombin this method will not show any FDP in serum from normal pregnant women who have a level of less than 5 g/l.

**Ethanol gelation test** according to Godal *et al.* (10).

**Antithrombin III** was determined immunochemically by the method of Fagerhol and Abildgaard (8). Normal value 98  $\pm$  7 per cent.

**Plasminogen** was measured by an immunochemical method according to Ekelund *et al.* (7). Normal value 121  $\pm$  0 per cent.

**Alpha<sub>2</sub> macroglobulin** was measured with the electrolytic method of Garrot (9). Normal value 134  $\pm$  4 per cent.

## RESULTS

Of the 73 consecutive cases of abruption placentae the fetus in four was dead on admission. All the patients were delivered by cesarean section. 67 in the acute

Table I Coagulation factors and components of the fibrinolytic system

Patient no	Thromb	Fib g/l	F VII C/ F VIII R Ag (%)	FV (%)	P&P (%)	APTT sec	Ethanol gelation	Plasminogen
1 MS	230 000	5.4	213/209	100	>200	36	negative	100
2 KBD	170 000	3.2	100/164	98	108	36	negative	95
3 MH	165 000	3.6	290/400	105	124	32	negative	120
4 IF		4.4	153/240	109	192	38	negative	130
5 IG	260 000	5.1	311/	107	117	30	negative	100
6 IN		5.1	130/236	131	160	40	negative	170
7 DSC			/					140
8 SA		3.0	195/180	103	97	36	negative	176
9 KP	230 000	2.8	220/137	85	89	40	negative	130
10 EL		4.4	203/248	93	172	32	negative	133
11 LJ		2.7	142/160	125	136	42	negative	80
12 BS	250 000	3.9	213/260	147	101	17	negative	80
13 MF		4.5	370/336	105	184	35	negative	150
14 AS		1.7	140/400	61	128	36	negative	60

Table I Continuation

AT III (%)	$\alpha_2$ macro (%)	Euglobulin precipitate (lysed area mm <sup>2</sup> )	FDP g/l
100	116	25	10
88	124	69	12
78	107	10	9
100	128	90	0
120	133	10	11
90	147		16
	113		39
70	106	36	76
112	114	130	11
80	122	46	0
60	137	38	0
90	149	49	0
135	175	10	13
55	75	31	60

stage and six after treatment with tranexamic acid for 1–12 weeks. Two neonates in the acute group were in a bad condition when delivered and did not survive, i.e. a total perinatal mortality of 8 per cent. The maternal mortality was nil. The clinical data on the six patients treated for a prolonged period with tranexamic acid are given in Table III. The neonates had an Apgar score of 8–9 at 60 sec after birth. No case of hemorrhagic diathesis, thrombotic complications or perinatal death occurred in this subgroup.

The values of coagulation factors and components of the fibrinolytic system examined in the acute stage in 14 of the patients are given in Table I. The concentration of fibrinogen was low in six cases. FDPs were found in blood in ten of the patients and low plasminogen levels were found in six. Four patients had somewhat low P&P levels and factor VIII activity and antigen were slightly depressed in some cases.

The ethanol gelation test was negative in all. Count factor V and AT III were within the range with the exception of case 14. The final follow up of two of the cases (9 and 12) is given during the treatment with tranexamic acid in Table II. During treatment FDP disappeared, the fibrinogen levels increased as did the concentration of plasminogen. No changes in the coagulation factors occurred.

## DISCUSSION

Of the 73 cases of abruptio placentae treated with tranexamic acid none were complicated by hemorrhagic diathesis and the perinatal mortality was 10 per cent. Of the 14 patients in whom coagulation factors and fibrinolytic components were measured at admission the concentration of fibrinogen and P&P complex were low in some, but the ethanol gelation test was negative and the platelet count was normal. The plasminogen levels were somewhat low and FDP occurred in most cases. These findings are not compatible with a marked intravascular coagulation or consumption coagulopathy but may be above all activation of the fibrinolytic system. This is also emphasized by the findings given in Table II, in which cases the concentrations of fibrinogen and plasminogen rose during treatment with tranexamic acid while FDPs disappeared. The results are in contrast to the conclusion of Sher *et al* (22) that hemorrhagic diathesis was due to consumption coagulopathy in 10 per cent of his cases of abruptio placentae. However, he also reported in resected

## Table II Coagulation factors and fibrinolytic components during therapy with tranexamic acid

no	Throm	Fib	F VII C/ F VIII R Ag	FV	P&P	APTT	Ethanol gelation
before treatment	230 000	2.8	220/137	85	89	40	negative
during treatment	410 000	3.6	175/155	92	196	38	negative
before treatment	250 000	4.5	240/138	60	90	39	negative
during treatment	225 000	3.9	213/260	147	101	37	negative
before treatment	245 000	4.7	130/212	170	92	38	negative
during treatment	245 000	5.0	400/292	174	136	35	negative

## Table II Continuation

no	AT III	$\alpha_2$ macro	Euglobulin precipitate	FDP
112	134	130	11	
110	125	51	17	
82	91	10	0	
90	149	49	0	
110	141	29	0	
110	154	10	11	

of FDPs in his cases. Edgington (6) used the age associated neo antigen of fibrinogen for differentiation between FDP derived from respectively fibrinogen and fibrin. In all his cases of abruptio placentae the FDPs were derived from fibrinogen, thus indicating above all activation of the fibrinolytic system.

During pregnancy fibrinolytic activity successively decreases and rapidly returns to its original level after delivery of the placenta. We have shown the significance of the placenta in depression of the fibrinolytic activity (25). The activation of the fibrinolytic system in these cases can be explained by release of plasminogen activators from the uterus which is known to be rich in such enzymes (1) together with placental inhibition by placenta when partly separated.

Sher (22) used the protease inhibitor Trasylol<sup>®</sup> and reported a good effect of such treatment. Astedt and Nilsson (26) have reported a case of recurrent abruptio placentae successfully treated with tranexamic acid.

Some authors feel that depression of fibrinolytic activity by administration of inhibitors during pregnancy might be hazardous (4, 23). For the apparently paradoxical non thrombogenic effect of tranexamic acid there are probably several explanations. In a series of patients receiving AMCA (tranexamic acid) for 2 weeks in a dose of 4 g a day we examined the fibrinolytic activity in vein biopsy specimens histologically before and on the last day of treatment. Tranexamic acid did not suppress the content of fibrinolytic activators in the vessel wall (unpublished data). Neither did any thrombotic complications occur in Klimek and Stanek (13) series of 92 patients with threatening abortion and treated for a long duration with epsilon aminocaproic acid.

The finding in the present investigation of an activation of mainly the fibrinolytic system speaks in favor of the use of a specific fibrinolytic inhibitor. Tranexamic acid given immediately might have prevented further activation of the fibrinolytic system since none of the patients developed hemorrhagic diathesis. We therefore believe that routine immediate treatment with tranexamic acid can reduce the perinatal mortality in cases of abruptio placentae.

## Table III Clinical data on the patients treated with tranexamic acid

no	Acute symptom of abruptio placentae (week of pregnancy)	Therapy with tran examic acid (weeks)	Cesarean section (week of pregnancy)	Apgar score (1 min etc)
35		3	38	8
26		12	38	9
32		1	33	9
33		4	36	9
27		10	37	8
29		5	34	9
Mean 30.2		Mean 5.8	Mean 36	Mean 8.7



## ACKNOWLEDGEMENTS

This work was supported by grants from the Council for Tobacco Research USA and the Swedish Medical Research Council (B79-17X-04523-05B and B79-19X-0087-15B)

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Submitted for publication November 6 1978

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# RISK OF SPONTANEOUS ABORTION FOLLOWING LEGALLY INDUCED ABORTION

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Using the registration of all pregnant women living in a particular geographical district of Copenhagen the risk of spontaneous abortion has been calculated by means of a decremental method. A total of 3 042 pregnancies were registered and the total risk of spontaneous abortion was calculated as 10 per cent. In 431 women the previous pregnancy had been terminated by a legally induced abortion. Increased risk of spontaneous abortion could not be demonstrated in this group where the cumulative risk of spontaneous abortion was 12 per cent. In particular the risk of spontaneous abortion was not seen to increase

The risk of spontaneous abortion was found to be related with the woman's age by Shapiro & Mowicz (9) and Petterson (6). Petterson (6) found the risk of spontaneous abortion minimal at 26 years and increased before and after this age. In addition the outcome of previous pregnancies were found to correlate with the risk of spontaneous abortion. Thus Petterson (6) as well as Warburton & Tr (11) found the risk of spontaneous abortion to be higher in women with previous spontaneous abortion than in others.

The reasons for spontaneous abortions are often abnormalities. Lauritsen (5) found chromosome abnormalities in 61 per cent in the first trimester. In many cases the reason must be sought in uterine malformations or in so-called cervical insufficiency. Since the mid sixties legal abortion is being increasingly used in a number of Western countries such as Denmark, Great Britain, Sweden and the USA, and so it is of even greater interest to study whether a legally induced abortion increases the risk of complications including the risk of spontaneous abortion in the next pregnancy.

On the basis of a register of all pregnant women in one geographical district the present study was conducted to evaluate if there is an increased risk of spontaneous abortion in women whose previous pregnancy was terminated by legal abortion.

## MATERIAL AND METHODS

The study included all women who delivered or aborted within the period 1st February 1975 to 31st January 1977 with a permanent address at Frederiksberg, an independent administrative unit in the center of Copenhagen. On 1st January 1976 there were 93 692 inhabitants including 16 857 women 15-45 years. This municipality was considered suitable for the study as the population figure was stable and the population was mainly referred to only one hospital.

The study is prospective and based upon data recorded in connection with abortion or delivery. In order to secure a complete record of all pregnancies data were collected in four different ways:

- 1 All women admitted to the local hospital for delivery or abortion were registered.
- 2 All deliveries in maternity homes were registered as it was possible to trace these women via the municipal accounts department.
- 3 All practitioners of this district reported on all pregnancies which did not lead to admission to the local hospital.
- 4 At the pharmacies in the district all women who had a positive pregnancy test received a letter asking them to cooperate in the study and to return a post-card enclosed stating name, address and telephone number. Eight women registered by the practitioners and 352 women registered by the pharmacies were then contacted on telephone or at their address if they were not registered by the local hospital. Through these two channels 11 women were registered who were not registered by the hospital at the same time.

In this study period 3 042 pregnancies were registered: 1 667 deliveries, 210 spontaneous abortions, 17 extrauterine pregnancies and 1 148 legal abortions.

On the basis of patient records and interviews the following data were recorded:

Date of delivery, previous pregnancies and their outcome, date of last menstrual period, date of the termination of the pregnancy, gestational age estimated by the size of uterus at the first gynaecological examination of the current pregnancy and the outcome of the pregnancy, whether delivery was spontaneous abortion, extrauterine pregnancy or legal abortion.

Spontaneous abortions were defined as registered pregnancies confirmed either by means of the presence of chorionic gonadotrophin or by histological examination which ended spontaneously before the end of 27th week of gestation with no signs of fetal movements.

Table I Number of registered pregnancies classified in age groups and pregnancy outcome: spontaneous abortion, extrauterine pregnancy, legal abortion or live birth. The table also shows the cumulative risk of abortion calculated by means of the decremental method

Age group	No of pregnancies	No of spontaneous abortions	No of extrauterine pregnancies	No of legal abortions	No of deliveries	Cumulative risk of abortion
< 21 years	349 100%	22 6.3%	1 0.3%	167 47.9%	159 45.5%	9.8%
21-30 years	1 938 100%	116 6.0%	11 0.6%	602 31.1%	1 209 62.3%	8.2%
> 30 years	755 100%	72 9.5%	5 0.7%	379 50.2%	299 39.6%	15.9%
Total	3 042 100%	210 6.9%	17 0.6%	1 148 37.7%	1 667 54.8%	10.0%

Cumulative risk significantly greater than among women in age group 21-30 years  $p < 0.05$

The gestational age at the termination of the pregnancy was calculated from the last menstrual period. In order to reduce the influence of an irregular menstrual pattern on the calculated gestational age the gestational age was also estimated by the size of the uterus at the first gynecological examination in the current pregnancy. In those cases where there was a difference of more than 8 weeks between the two methods calculations of gestational age were based on the size of the uterus.

The woman's age was recorded as her age at the time of conceiving.

The total patient data were classified according to the termination of the previous pregnancy and the following four categories were described:

Group 1: Women whose previous pregnancy was terminated by a legal abortion: 431 women.

Group 2: Women whose previous pregnancy had ended in spontaneous abortion or extrauterine pregnancy: 254 women.

Group 3: Women whose previous pregnancy had ended in a delivery: 1 171 women.

Group 4: Women with no previous pregnancies: 1 186 women.

The sum of spontaneous abortions and extrauterine pregnancies was taken as the calculated risk of spontaneous abortion. This risk was calculated by means of a decremental method. All pregnancies within the study were considered as being under observation from the date of conception.

The risk of spontaneous abortion in each group was compared by means of a generalized edition of Wilcoxon's test, the so-called Gehan's test described by Gehan (3). 0.05 was regarded as the significance level.

## RESULTS

Table I shows the results classified according to age groups: below 21 years, 21-30 years and over 30 years. The table indicates the distribution of pregnancy outcome as well as the cumulative risk of spontaneous abortion in the three age groups calculated by means of the decremental method. There was no difference in abortion risk between the two younger

age groups (9.8 and 8.2 per cent) whereas the spontaneous abortion was greater over 30 years per cent.

Table II shows the risk of spontaneous abortion: the total number of patients calculated by the decremental method. The cumulative risk was 10.0 per cent. Table III has the equivalent group I. The cumulative risk of spontaneous abortion was 12.0 per cent. In addition, table III shows 431 pregnant women of this group: no abortions were registered after 18 weeks of pregnancy.

Table IV shows the cumulative risk of abortion in groups 1 to 4 in the age groups: below 21 years and over 30 years. As the number of women in the group under 21 years was too small the risk of spontaneous abortion in each of the groups separately.

In the age groups 21-30 years and over 30 years there was no difference in the risk of abortion between group 1 and groups 2-4. Correspondingly, no difference in risk of spontaneous abortion could be demonstrated between groups 1-2 and 3 in women with only one previous pregnancy and neither when women in group 1 only one previous pregnancy were compared with women in group 4.

## DISCUSSION

This study demonstrated a cumulative risk of spontaneous abortion of 10.0 per cent. The risk was greatest in women over 30 years of age: 15.9%. The risk of spontaneous abortion was calculated by means of the decremental method. We have previously adopted this method to calculate the risk of a pregnancy not ending in a live birth (York 1958, 1960; Shapiro and Abramowitz

II Risk of spontaneous abortion in women living within the district of Frederiksberg pregnant in the period February 1975 to 31st January 1977

No of spontaneous abortions	No of induced abortions	Pregnant at beginning of the week	Exposed to risk of spontaneous abortion	Risk of spontaneous abortion	Cumulative risk of spontaneous abortion	Standard deviation
3	1	3 042	3 041.5	0.10	10.0	0.6
11	10	3 038	3 033.0	0.36	9.9	0.6
21	22	3 017	3 006.0	0.70	9.6	0.6
22	90	2 974	2 929.0	0.75	8.9	0.6
14	252	2 862	2 736.0	0.51	8.2	0.6
25	265	2 596	2 463.5	1.01	7.8	0.6
23	225	2 306	2 191.5	1.05	6.8	0.6
23	131	2 058	1 992.5	1.15	5.8	0.5
23	74	1 904	1 867.0	1.23	4.7	0.5
15	25	1 807	1 794.5	0.84	3.5	0.4
12	24	1 767	1 755.0	0.68	2.7	0.4
5	8	1 731	1 727.0	0.29	2.1	0.3
5	7	1 718	1 714.5	0.29	1.8	0.3
10	6	1 706	1 703.0	0.59	1.5	0.3
1	3	1 690	1 688.5	0.06	0.9	0.2
1	4	1 686	1 684.0	0.06	0.8	0.2
1		1 681	1 681.0	0.06	0.8	0.2
3	1	1 680	1 679.5	0.18	0.7	0.2
1		1 676	1 676.0	0.06	0.5	0.2
1		1 675	1 675.0	0.06	0.5	0.2
2		1 674	1 674.0	0.12	0.4	0.2
3		1 672	1 672.0	0.18	0.3	0.1
2		1 669	1 669.0	0.12	0.1	0.1

of 15 per cent of a pregnancy not ending in a birth and Taylor (10) found in 1959-1960 in a pregnancy loss of 16 per cent. In 1960 in a from New York in which the risk of spontaneous abortion within the first weeks of gestation was stated by extrapolation. Erhardt (2) found a pregnancy loss of 29.5 per cent. In Sweden Pettersson and a risk of spontaneous abortion of 14 per cent up to 27 weeks gestation.

Our study included all pregnant women living in a certain district of Copenhagen. All women registered at the termination of their pregnancy. Risk of spontaneous abortion was calculated in a way that all women entered into the analyses at the date of conceiving. So the present results cannot be compared directly with the above mentioned studies in which a woman was admitted into the analyses only from the moment the pregnancy was lost. This particularly leads to a difference in calculated risk of spontaneous abortion during the first weeks of pregnancy when the deficit between the number of lost pregnancies and the number of registered spontaneous abortions must be expected to be greatest. The reason is that abortions in such an early date do not always make the women consult her doctor who would be able to make the diagnosis and

also that such early spontaneous abortions will often not be recognized as an abortion by the woman herself. The calculated risk of spontaneous abortion during the first weeks must therefore be considered with reservations and taken as the minimum rate. Only Erhardt's study (2) using extrapolation tried to estimate the pregnancy loss during the first weeks of pregnancy and his study reported a high estimate of pregnancy loss.

Another factor which makes the calculation of risk of spontaneous abortion inaccurate is that some pregnancies are terminated by illegal abortion. All the above studies were made in countries where abortion was not legalized; therefore an unknown number of pregnancies would probably have been terminated by illegal abortion. In all probability some illegal abortions would be registered as spontaneous abortion making the calculated spontaneous abortion risk too high. In Denmark all women have the right to have an unwanted pregnancy terminated before 12 weeks gestation so the number of illegal abortion will presumably be small. However a situation of this kind may lead to another source of error. A calculation of the risk of spontaneous abortion is based upon an indirect presumption that the pregnant women entering into the analyses at any time are a

Table III Risk of spontaneous abortion in 431 women whose previous pregnancy had been legal abortion

Week	No of spontaneous abortions	No of induced abortions	Pregnant at beginning of the week	Exposed to risk of spontaneous abortion	Risk of spontaneous abortion	Cumulative risk of spontaneous abortion	dev
5			431	431 0		12 0	2 0
6		2	431	430 0		12 0	2 0
7	6	7	429	425 5	1 41	12 0	2 0
8	4	11	416	410 5	0 97	10 7	2 0
9	2	51	401	375 5	0 53	9 8	2 0
10	3	48	348	324 0	0 93	9 3	2 0
11	4	36	297	279 0	1 43	8 5	1 9
12	2	30	257	242 0	0 83	7 2	1 8
13	3	15	225	217 5	1 38	6 4	1 7
14	3	5	207	204 5	1 47	5 1	1 6
15	4	7	199	195 5	2 05	3 7	1 4
16		3	188	186 5		1 6	0 9
17		2	185	184 0		1 6	0 9
18	3		183	183 0	1 64	1 6	0 9
19							
20							
21							
22							
23			180	180 0			
24							
25							
26							
27							

representative group. This presumption will be correct only if the women with legal abortions have the same risk of spontaneous abortion as those whose pregnancy is continuing. It is difficult to say to what extent this presumption is correct. Women admitted to hospital with a threatened abortion and thus a high risk of spontaneous abortion may for instance choose to have an unplanned pregnancy terminated as a legal abortion rather than submit to prolonged confinement to bed. Such a situation would result in an underestimate of the risk of spontaneous abortion.

This study particularly tried to evaluate whether there was an increased risk of spontaneous abortion in women whose previous pregnancy had been terminated by a legal abortion. The background of this theory was based upon two factors. Firstly a presumption that dilatation of the cervical canal would result in lesions causing an increased risk of cervical insufficiency. Wright *et al* (12) demonstrated an increased risk of cervical insufficiency in women with a previous legally induced abortion. The second factor was that intrauterine changes such as a defective uterine cavity and synechia as may be seen after a legal abortion. Kralj & Lavric (4) and von Seewald (8) might impede nidation and placental function

with an ensuing risk of spontaneous abortion.

The present study could not demonstrate an increased risk of spontaneous abortion in women whose previous pregnancy had been terminated by a legal abortion. The cumulative risk of abortion in this group was 12.0 per cent in the age group 21-30 years nor in women over 30 years. The risk of spontaneous abortion significantly increased in women whose previous pregnancy had been terminated by a legal abortion when compared with women whose previous pregnancy had ended in a delivery or who had not been pregnant before. Table IV. It is of particular interest that after a legal abortion there were no spontaneous abortions more than 18 weeks of gestation in the present study (Table III). This finding does not support the theory that legal abortion causes an increased risk of cervical insufficiency; therefore we are not able to confirm the findings of Wright *et al* (12). Our results are consistent with the findings of Daling & Emswiler (13) who did not demonstrate an increased risk of spontaneous abortion in women with a previous legal abortion. In a retrospective study from Japan, Kato & Aoyama (7) demonstrated an increased frequency of spontaneous abortions in pregnancies following a legal induced abortion as did Petterson (6). Most

Table IV Cumulative risk of spontaneous abortion among women whose previous pregnancy ended in legally induced abortion group 1 spontaneous abortion group 2 delivery group 3 and women without previous pregnancies group 4

	All pregnancies				Women with only one previous pregnancy		
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3
at conception 21-30 years							
no of previous pregnancies	n	n	n	0	1	1	1
no of pregnancies registered	245	153	738	802	110	71	458
no of spontaneous abortions	17	19	46	45	8	7	30
cumulative risk of spontaneous abortion	9.6%	13.3%	7.8%	7.1%	6.9%	10.1%	7.9%
at conception > 30 years							
no of previous pregnancies	n	n	n	0	1	1	1
no of pregnancies registered	152	90	391	122	24	24	108
no of spontaneous abortions	16	15	33	13	3	3	10
cumulative risk of spontaneous abortion	21.4%	21.6%	14.3%	12.8%	15.7%	15.0%	12.6%

> 0

tions in Petterson's study were however performed by vaginal hysterotomy so his results do not compare directly with the results of this study made in Denmark where 9 per cent of all induced abortions performed by dilatation and curettage 82 per cent by the suction method and vaginal hysterotomy rarely used.

## CONCLUSIONS

In a population living within a well defined district of Copenhagen we found a cumulative risk of spontaneous abortion of 10 per cent by means of the suction method. Related to age the risk was significantly greater in women over 30 years of age 15.9 per cent than in women of the age group 21-30 years 9.6 per cent. The risk of spontaneous abortion was not increased in 431 women whose previous pregnancy was terminated by a legal abortion. The cumulative risk of spontaneous abortion was 12.0 per cent. It is particularly interesting that in this group there were no spontaneous abortions after 12 weeks gestation.

## ACKNOWLEDGEMENTS

This study has received financial support from the Danish Medical Research Council.

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Submitted for publication July 27 1978

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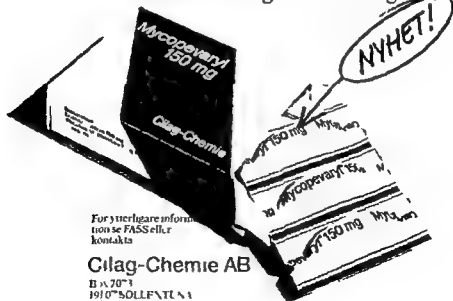


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## HYPERPROLACTINEMIA IN CASES OF INFERTILITY AND AMENORRHEA

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**Abstract** Of 17 patients with longstanding (3-15 years n 7 7 years) amenorrhea and hyperprolactinemia 8 developed their amenorrhea after the use of oral contraceptives (Group I) and 9 became amenorrhoeic spontaneously (Group II). There were no differences between the groups with respect to the basal serum levels of FSH, LH, low polar estrogens (estradiol 17 $\beta$  + estrone) and prolactin. Tomography revealed pituitary adenoma in four patients. One of the developed symptoms of her tumor during pregnancy and symptoms disappeared after delivery. The other patients' tumors are checked twice a year and have not yet required any treatment. The patients with no detectable tumors were treated with bromocriptine starting with 1.25  $\times$  daily. The peripheral serum levels of prolactin, FSH, LH, low polar estrogens and progesterone were determined once a week and if the prolactin levels remained high the bromocriptine dose was increased. All these patients started to menstruate as soon as prolactin returned to normal levels within 1-2  $\mu$ g/l. All patients who wished to become pregnant 5 patients. Three were delivered by caesarean section, one had a normal delivery and two are still pregnant. There was no difference between Group I and Group II in the time required or in the duration of treatment before menstruation started. Three cases of galactorrhea were treated.

Hwang and co-workers (7) described the first method for radioimmunological determination of prolactin in peripheral human circulation. Since then the knowledge on the physiology of prolactin has increased immensely. The frequency of hyperprolactinemia is high among women suffering from amenorrhea and anovulation. Galactorrhea is not always present in these cases. It is supposed that hyperprolactinemia disturbs the normal menstrual cycle by interfering with the hypothalamic level with the positive feedback of estrogens upon the release of gonadotrophins. Women with amenorrhea due to hyperprolactinemia have no episodic LH secretion and do not respond to clomiphene treatment. It has been found that about 30 per cent of the pituitary adenomas earlier considered to have no function are associated with a hypersecretion of prolactin (4). These tumors are often difficult to diagnose if the visual fields are normal and so is the X-ray picture of the skull and sella turcica. It is necessary to

complete the examination with tomography. The frequency of such tumors in cases of hyperprolactinemia has been discussed extensively but at least 30 per cent of the women with high prolactin levels have a pituitary adenoma (10). Bromocriptine (2-bromo- $\alpha$ -ergocryptine), a long-acting dopamine receptor agonist, decreases prolactin levels. Thus it is possible to restore the function of the ovaries and normal menstruations occur as long as the treatment continues. Since the first report by Besser and co-workers in 1972 (2) concerning treatment of amenorrhea and infertility with bromocriptine, more than 100 reports have been published on this subject. In Sweden Bergh and co-workers (1) recently published a material of 42 women with longstanding amenorrhea who had been treated with bromocriptine. Among them 24 had radiological signs of a pituitary tumor. Twenty-one out of 22 women who wanted to become pregnant conceived. The present communication describes the results of treatment with bromocriptine in patients with spontaneous and post-pill longstanding amenorrhea respectively.

## MATERIAL AND METHODS

**Clinical Material** For a long time now the routine investigation of patients with longstanding amenorrhea has included determination of peripheral serum levels or urinary excretion of gonadotrophins and estradiol 17 $\beta$  + estrone (LPE, low polar estrogens). The patients had been examined once a year with hormone determinations and with respect to visual fields. After the introduction of prolactin determinations in 1976 17 patients with amenorrhea lasting for more than 3 years were found to have high serum levels of prolactin (>25  $\mu$ g/litre). Eight patients (Group I, Table I) developed their amenorrhea when they stopped taking combined oral contraceptives. Nine patients (Group II, Table II) had no demonstrable reason for their amenorrhea. All patients were euthyroid.

The patients were treated with bromocriptine (Pramifex<sup>®</sup>, Sandoz A.G.) starting with 1.25 mg  $\times$  3 daily. The serum levels of prolactin, FSH, LH, LPE and progesterone were determined once a week and in cases where prolactin levels remained high the dose of bromocriptine was increased.



Table 1 Post pill amenorrhea (Group I)

Case	Age	Duration of amenorrhea Years	X ray sella	Tomography	Visual fields	FSH U/l	LH U/l	LPE pM	Prolactin ng/l
1	45	7 <sup>a</sup>	normal	normal	normal	4.2	7.8	172	< 20
2	42	12	normal	normal	normal	7.6	4.0		> 20
3	40	3 <sup>b</sup>	normal	tumor	normal	4.1	15.5	108	> 20
4	37	8	normal	normal	normal	4.2	7.9	130	> 20
5	33	11	normal	normal	normal	18.5			161
6	30	6	normal	normal	normal	8.1	8.5	134	> 20
7	28	5	normal	normal	normal	2.5	5.1	85	> 20
8	28	11	normal	normal	normal	3.9		78	80
Mean $\pm$ S.D.						6.6 $\pm$ 5.1	8.1 $\pm$ 4.0	117.8 $\pm$ 34.9	

a) Galactorrhea b) Galactorrhea Poor response upon LH RH stimulation

### Hormone analyses

Serum prolactin was determined by using a radioimmunoassay kit from CEA IRESORIN (Fleurus Belgium). The values are expressed as  $\mu$ g NIH (1) prolactin per litre. Serum levels of FSH and LH were determined by radioimmunoassay using the Double Antibody Solid Phase (DASP<sup>(R)</sup>) Organon OSS Holland separation technique. Antibodies against FSH and LH as well as highly purified FSH and LH for iodination were obtained from KABI AB Stockholm Sweden. The <sup>125</sup>I labelling was performed by the chloramine T technique described by Hunter & Greenwood (6). The values are expressed as units per litre of Human Pituitary FSH 68/39 and Human Pituitary LH or ICSH 68/40 respectively.

Serum progesterone was determined radioimmunologically after extraction with n-hexane (11). Anti progesterone 11 $\alpha$  hemisuccinate bovine serum albumin (sheep) was obtained from The Royal Veterinary College Uppsala Sweden.

Serum levels of low polar estrogens (LPE) were determined by the radioimmunoassay technique of Edqvist & Johansson (3) using an antiserum against estradiol 17 $\beta$  hemisuccinate bovine serum albumin (8). This antibody reacts in 100 per cent with estradiol 17 $\beta$  and to 50 per cent with estrone. The values are expressed as pmol immunoreactive estradiol equivalents per litre.

The intra and inter assay variations of the methods for prolactin 5.0 and 7.2 per cent for FSH 8.0 and 11.1 per cent for LH 8.7 and 9.4 per cent for progesterone 11.1 and 11.5 per cent and for low polar estrogens 13.1 and 11.1 per cent.

### RESULTS

The basal hormone values for Groups I and II are given in Tables I and III. There were no significant differences between the mean hormone values of the two groups. The values for FSH and LH corresponded to those found during the follicular phase of the menstrual cycle while the values for LPE were in the same range as for postmenopausal women. Prolactin was elevated ( $>25 \mu$ g/l) in all cases. In Group I (Table I) two women, 45 and 47 years of age (patients 1 and 2) refused treatment with bromocriptine. They are being treated cyclically with estrogens and gestagens and checked twice a year for terminations of the visual fields and once a year for tomography of the sella. One patient (No 3) had a pituitary tumor and also responded poorly to the

Table II Results of treatment with bromocriptine (Group I)

Case	Age	Dose	Duration <sup>1)</sup>	Comments
1	45			Cyclic treatment with estrogens + progesterone
2	42			Cyclic treatment with estrogens + progesterone
3	40			Tumor not yet treated
4	37	1.25 $\times$ 3 $\times$ 3 mo 2.5 $\times$ 4 $\times$ 2 mo	5 mo	
5	33	1.25 $\times$ 3	1 mo	
6	30	1.25 $\times$ 3 $\times$ 2 mo 2.5 $\times$ 3 $\times$ 3 mo 2.5 $\times$ 4 $\times$ 2 mo	7 mo	
7	28	1.25 $\times$ 3	1 mo	Stopped medication. Menses disappeared. Started again. Menstruation returned. Pregnant, delivered in the 40th week.
8	28	1.25 $\times$ 3	1 mo	Pregnant after 2 months of treatment. Delivered in the 40th week.

) Dose of bromocriptine in mg 1) Duration in months before the first menses

## Table III Spontaneous amenorrhea (Group II)

Age	Duration of amenorrhea Years	X ray sella	Tomography	Visual fields	FSH U/l	LH U/l	LPE pM	Prolactin µg/l
46	3	normal	normal	normal	13.7	11.0	179	>200
39	3.5	suspect	tumor	normal	5.2	9.6	81	50
33	12	normal	normal	normal	1.3	9.9	78	>200
33	8 <sup>b</sup>	empty	tumor	normal	0.8	6.0	32	106
9	8	normal	normal	normal	6.7	10.0	39	98
29	14	normal	tumor <sup>f</sup>	normal	2.9	11.3	52	>200
25	7 <sup>d</sup>	normal	normal	normal	9.7	14.5	110	>200
22	5	normal	normal	normal	4.7	9.4	55	107
21	5	normal	normal	normal	7.2	8.3		>200
±SD 30.8±8.1 7.3±3.7					5.8±4.1	10.0±2.3	78.4±48.0	

a) Response on LH RH stimulation b) No response on LH RH stimulation c) Poor response on LH RH stimulation Galactorrhea d) Refused treatment e) Detected during pregnancy

ulation. The X ray of the sella was negative but tomography revealed an enlarged sella turcica. No comment has yet been given. The other patients (Table II) responded to the treatment with normal menstrual cycles. Two of them (No. 7 and 8) wanted to conceive after 2 and 3 months of treatment respectively. They were delivered in the 30th week of pregnancy, patient 7 with cesarean section.

Table II In patient 10 (Table III) tomography revealed an asymmetrically enlarged sella which had slightly increased at re-examination three months later. She is being treated with bromocriptine regularly and the visual fields are normal. Patient 11 was examined in 1975 and then did not respond to LH RH stimulation. She was therefore examined at the Neurological Department of Karolinska sjukhuset, Stockholm. No changes in the sella were then detected. Upon re-examination two years later the serum prolactin levels were determined and found to be

elevated. The sella was now examined with tomography and cisternography which gave evidence of a tumor without any supra-cellular spread. The patient feels well and is not being treated. She is examined every third month. Patient 15 refused treatment because she did not believe in new drugs and did not want to be an experimental animal. She is checked twice a year.

All patients in this group who wanted to be pregnant conceived (No. 13, 14, 16, 17). Patient 13 aborted in her 12th week of pregnancy but got pregnant again one month later. She is now in her sixth month of pregnancy. Patient 16 is pregnant in the 20th week. Patient 14 had galactorrhea now and had previously been treated 5 times with hCG and hMG. She responded to that treatment with ovulation but did not get pregnant. Prior to treatment with bromocriptine the visual field and sella X ray examination yielded normal results. She started to menstruate after one month of treatment and conceived after two months.

## Table IV Results of treatment with bromocriptine (Group II)

Age	Dose	Duration <sup>f</sup>	Comments
46	1.25 × 3	3 we	Tumor
39	1.25 × 3	1 mo	
33	1.25 × 3 × 6 we 2.5 × 3 × 3 mo	4.5 mo	
33	—	—	Tumor, no treatment
29	1.25 × 3	1 mo	Pregnant, Aborted in 12th week. Pregnant again
25	1.25 × 3	1 mo	Pregnant, Delivered in the 36th week. Tumor was found
25	—	—	Refused treatment
29	1.25 × 3	9 we	Pregnant
21	1.25 × 3	2 mo	Pregnant, Delivered in the 36th week. Placenta previa

we = weeks of bromocriptine in mg; f) Duration in week or months before the first menses

Her visual fields were examined once a month. She complained of headache from the third month of pregnancy and in the sixth month defects in the visual fields appeared. Bromocriptine treatment (10 mg daily) was then started. A tomography in the 34th week showed that dorsum sellae was very thin, which could be explained by an intracellular expansive process. In spite of the bromocriptine treatment the defects in the visual fields became enlarged, the headache became worse and the sight of the left eye diminished considerably. She was therefore delivered in the 36th week of pregnancy by caesarean section. After the delivery the headache and the defects in the visual fields disappeared within 10 days and her sight became normal. She stopped the bromocriptine treatment 1 month after delivery and started to breastfeed her baby. Patient 17 had a placenta previa and was therefore delivered in the 36th week of pregnancy with caesarean section.

### DISCUSSION

The clinical material in this study included only patients who had been amenorrhoeic for more than 3 years (mean  $7.7 \pm 4.2$  range 3–15) and who showed elevated prolactin but normal FSH levels. In this small group we found 4 cases, i.e. 23.5 per cent, who had a pituitary tumor. These patients should be carefully monitored during pregnancy and have their visual fields checked frequently because there is a risk that such tumors may grow rapidly during the last trimester, inflicting damage to the optic nerve. Such changes occurred in one of our patients but they disappeared almost immediately after delivery.

The frequency of hyperprolactinemia among all our cases of amenorrhoea is difficult to evaluate as we have determined the prolactin levels in all new patients but also among our cases with longstanding amenorrhoea who are re-examined once a year at our department. Longstanding post-pill amenorrhoea has been attributed earlier to a persistent disturbance in the hypothalamus as a result of the pill intake. One hundred and seventy-seven patients with post-pill amenorrhoea have previously been examined endocrinologically and followed up by us (5). Spontaneous cure followed in almost 90 per cent of the cases within 5 years. Among the patients who still were amenorrhoeic 8 patients lived in Stockholm and could be re-examined. All of them had hyperprolactinemia and one of them a pituitary adenoma. It is interesting to speculate if the hyperprolactinemia could be a

result of the treatment with contraceptive pills; conflicting results have been presented in the literature (for references see 1). However, among our cases of spontaneous amenorrhoea 9 patients with longstanding amenorrhoea (>3 years) associated with hyperprolactinemia and 3 of them had a pituitary adenoma. Furthermore, there were no differences in the gonadotrophin and estrogen levels between the two groups. This speaks in favour of other factors than oral contraceptives as a cause of the hormonal disturbance.

All patients who were treated with bromocriptine started to menstruate and all of them who were not became pregnant. It is well known that when treatment with bromocriptine is stopped, the high elevation of prolactin and the amenorrhoea return. The question arises if patients who do not want to become pregnant have to be treated. If they start to menstruate they have of course to use some kind of contraception. Provided the patients are checked regularly for a possible pituitary adenoma, it may be better to substitute the lack of ovarian hormone with genuine estrogens and progesterone. This treatment, on the other hand, is not without risk, as it is known that pituitary tumors grow under the influence of estrogens. Among our patients 2 wanted to stop the bromocriptine treatment and have substitution therapy instead.

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*Submitted for publication November 10 1978*

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## ANNOUNCEMENT

The 11th International Congress of Psychosomatic Obstetrics and Gynecology will take place in West Berlin September 2-6 1980. The principle theme of the Congress — *Women in a changing society* — is meant to encourage lectures and discussions dealing with socio-psychological characteristics and cultural influences, psychosomatic symptoms and illness as well as with the psychotherapeutic potential in obstetrics and gynecology.

The Congress program includes plenary lectures delivered by invited speakers as well as short lectures on recent psychosomatic findings. In addition to those working groups, scientific films and demonstrations are being planned.

### *Languages of the Congress*

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### *Information may be received from*

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An International Symposium on Carcinoma of the Uterus will be held on Kiawah Island, Charleston, South Carolina, USA, September 4-7, 1980.

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The First International Symposium on Recent Prenatal Diagnosis will be held at the Pre-eclampsia Congress in Bologna, Italy, on the 15th to 18th September 1980. The symposium, organized by the Medical and Gynecological Department of the Bologna University and by the F. Angelini Research Institute, will include a number of Round Tables on prenatal diagnosis.

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## AN ANALYSIS OF THE INTENSITY AND QUALITY OF GYNECOLOGICAL PAIN

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It is evident that the pain experience varies as well as in intensity. A number of methods to both the intensity and quality of pain have been described. This paper presents the results of a card sort method of pain assessment in the measurement of gynecological pain which provides a score from 1 to 4 for 10 qualities. Factor analysis has identified 3 main clusters: dimensions, sensations, reaction and a turning/duration dimension.

Comparative data is presented on 5 main pain types using the method: dysmenorrhea, IUD related pain, IUD insertion, postoperative pain and post partum pain. A total of 100 patients were assessed. The results permit comparison between pain types for both intensity as well as quality distinction may have implications for pain management. Thus although dysmenorrhea and IUD related pain similar in terms of sensations, dysmenorrhea was rated significantly higher in terms of the reaction component. Similarly both IUD insertion pain and post partum pain had high reaction scores. The implications of such a profile are discussed. Thus these scores may reflect the unexpected nature of the pain sensations rather than their high intensity. In which case inclusion of psychological preparation may have been of benefit. Relief may be provided by focussing on the reaction component where this is elevated and removing the worry inducing sensations.

Without identifiable organic cause is a common presenting complaint in gynecological clinics. As reported by (9) comments: 'pain occurring below the umbilicus in the female abdomen is probably one of the most common presenting symptoms in gynecology'. The task of the gynecologist is both the diagnosis so that treatable organic pathology is excluded and effective management of the pain. In order to achieve this suitable methods of pain assessment must be available as it is necessary to rely upon the patient's verbal description of pain for both diagnosis and subsequent evaluation of treatment response. It is evident that pain varies qualitatively as well as in intensity (variation which needs to be reflected in pain measures so that changes in either intensity or quality over time are apparent). Thus Beecher (3) stressed

that the pain experience is dependent upon both the original sensations and the psychic processing of this sensation i.e. the reaction or evaluative component of pain. The way in which psychological processes such as mood, personality and culture can affect the reaction to similar sensations is well documented (20). Attempts to measure pain should take account of this evidence as information will be lost by relying on rating scales which treat pain as a single sensory quality varying only in intensity.

In view of the need to measure the quality as well as the intensity of pain over time a number of multidimensional assessment techniques have been described. Melzack (11) presented a questionnaire which provides scores on three main dimensions: sensory, affective and evaluative. Alternatively combinations of rating scales have been used to independently assess the sensory and distress/reaction components of pain (8, 10). A card sort method which provides a range of scores from 1 to 4 for 10 pain qualities has also been described (19). An evaluation of this method found it to have high reliability and concurrent validity in that sort scores were closely related to other pain indices such as taking pain killers, going to bed etc (18).

This paper presents the results of the routine use of this card sort method in inpatient and outpatient gynecological settings. Information on intensity ratings for a range of pain qualities will be presented for 5 types of pelvic pain: dysmenorrhea, intrauterine device related pain, post IUD insertion pain, postoperative pain and postpartum pain. The aim in supplying this information is to aid the physician in his understanding of patient's pain complaints.

## MATERIAL AND METHODS

Over the course of an 11 month period a total of 232 patients have been seen for a pain assessment. The card sort is in the form of paired comparisons. There are 10 qualities or triads each consisting of three words reflecting different amounts of qualitatively similar pain experience (Table I). For example the words pulsing, throbbing, pounding refer

Table I *Pain card sort assessment content*

Triad number	Intensity		
	i	ii	iii
1	pulsing	throbbing	pounding
2	dull	aching	heavy
3	pulling	cramping	wrenching
4	pricking	boring	stabbing
5	nagging	sickening	agonising
6	slightly tiring	tiring	exhausting
7	brief	every now and then	constant
8	annoying	miserable	unbearable
9	mild	discomforting	excruciating
10	slightly distressing	distressing	very distressing

Table II *Mean age according to pain group*

Pain group	Mean age	No
Dysmenorrhea	24.4 (8.0)	102
IUD related pain	24.2 (5.3)	38
Post insertion	23.7 (5.4)	22
Postoperative	30.3 (4.9)	55
Postpartum	24.0 (8.4)	15

Standard deviations in brackets

to different amounts of a similar pain sensation and represent one of the 10 triads. The content of the test was drawn from a survey of over 200 women complaining of pelvic pain (16). Only words within a triad are compared which means as there are 3 cards for each triad there are 30 cards in the present form of the test. The method of paired comparisons permits the internal consistency of each administration to be established.

## RESULTS

The number of patients in each of the pain groups shown in Table II along with their mean age are presented for Caucasian patients only as the present form of the card sort was developed for use in this ethnic group and may not be directly applicable to others in view of the documented variations of pain descriptors across ethnic groups (7).

In Table III a distinction is made between scores of 1 and 2 (minimal and mild) and 3 (moderate and severe) and the percentage of patients within each pain group achieving these scores is presented. Chi Square analyses of associations between scores and pain types for each pain triad

Table III *Percentage of patients scoring high (3-4) or low (1-2) on each triad of the card sort according to pain group*

Triad number	Score	Dysmenorrhea (%)	Pain IUD related (%)	Postinsertion (%)	Postoperative (%)	Postpartum (%)	Significant of $\chi^2$
1	low	53	55	81	45	67	$p < 0.5$
	high	47	45	17	35	33	
2	low	54	61	55	61	78	NS
	high	48	39	45	39	22	
3	low	71	68	77	63	56	NS
	high	29	32	23	37	44	
4	low	33	29	46	53	45	NS
	high	67	71	54	47	55	
5	low	64	88	65	87	67	$p < 0.1$
	high	38	12	35	11	32	
6	low	48	84	73	80	56	$p < 0.1$
	high	52	16	27	20	44	
7	low	36	54	81	41	66	NS
	high	64	46	19	59	46	
8	low	52	79	74	95	67	$p < 0.1$
	high	38	21	26	5	33	
9	low	47	66	59	88	45	$p < 0.1$
	high	53	36	41	12	55	
10	low	60	79	69	95	56	$p < 0.1$
	high	40	35	31	5	44	

Table IV Mean total and factor scores on card sort according to pain group

group	No	Card sort total	Sensation factor score	Tiring/temporal factor score	Evaluative factor score
dysmenorrhea	102	25.2 (6.9)	7.3 (2.2)	5.5 (1.7)	9.8 (3.8)
IUD related pain	11	22.0 (6.4)	7.6 (2.3)	4.4 (2.3)	7.3 (3.6)
postinsertion	22	22.0 (6.3)	6.1 (2.1)	4.6 (1.4)	8.2 (3.7)
postoperative	55	19.6 (6.2)	5.8 (2.9)	4.4 (2.2)	5.8 (2.6)
postpartum	15	21.5 (7.4)	7.1 (2.3)	5.0 (1.6)	9.3 (1.5)

Standard deviation in brackets

significant associations for triad 5 ( $\chi^2=15.8$   $df=4$   $p<0.01$ ) triad 6 ( $\chi^2=21.2$   $df=4$   $p<0.01$ ) triad 8 ( $\chi^2=24.3$   $df=4$   $p<0.01$ ) triad 9 ( $\chi^2=27.9$   $df=4$   $p<0.01$ ) and triad 10 ( $\chi^2=24.0$   $df=4$   $p<0.01$ ). With the exception of triad 1 ( $\chi^2=12.2$   $df=4$   $p<0.05$ ) significant associations between pain quality and pain group are found for triads describing the sensory and evaluative nature of the pain. Less association between groups is found for sensation quality.

Mean summated card sort scores and factor scores were calculated and are shown in Table IV. Factor analysis showed a priori clusters of triads to be condensed with three main factors emerging (18). These related to sensations (triads 1, 3 and 4), the evaluation of the pain (triads 5, 8, 9 and 10) and a third factor relating to the tiring/temporal dimension (triads 6 and 7). Associations between mean card sort totals and mean scores on each factor were assessed by analysis of variance. It was found that total card sort score was significantly associated with pain type ( $F=5.4$   $df=4$   $p<0.01$ ). Similarly factor scores were significantly related to type of pain: sensation ( $F=4.9$   $df=4$   $p<0.01$ ), tiring/temporal ( $F=4.6$   $df=4$   $p<0.01$ ) and evaluative ( $F=10.0$   $df=4$   $p<0.01$ ).

The significance of paired comparisons between pain groups were assessed by double tailed *t* tests in order to identify the source of this significant between group variation. It can be seen from Table IV that dysmenorrhea was assigned the highest score overall. This was significantly higher than the totals for the other pain groups (Dysmenorrhea and IUD related pain  $p<0.01$ ; Dysmenorrhea and postinsertion pain  $p<0.01$ ; Dysmenorrhea and postoperative pain  $p<0.01$ ; Dysmenorrhea and postpartum pain  $p<0.05$ ).

The significance of factor score differences between pain groups was also assessed. Comparison of dysmenorrhea with IUD related pain showed signifi-

cantly higher scores on the evaluative ( $p<0.01$ ) and tiring/temporal ( $p<0.001$ ) components for the dysmenorrhea group. Sensory factor scores were similar for both groups. Dysmenorrhea was reported to be significantly more painful than postinsertion pain in terms of both sensations ( $p<0.05$ ) and tiring/temporal quality ( $p<0.05$ ) although the evaluative score did not differ significantly. In comparison with postoperative pain, dysmenorrhea was rated as significantly more severe on all three components: sensation ( $p<0.001$ ), tiring/temporal ( $p<0.001$ ) and evaluative ( $p<0.001$ ). No significant differences between dysmenorrhea and postpartum pain emerged.

A comparison of IUD related pain with post IUD insertion pain showed higher sensation ratings for IUD related pain ( $p<0.05$ ) with no significant difference in terms of either tiring/temporal or evaluative dimensions. IUD related pain was reported to be significantly more painful in terms of both sensations ( $p<0.01$ ) and evaluation ( $p<0.01$ ) than postoperative pain. No significant differences between IUD related and postpartum pain occurred.

Post insertion pain was rated as significantly more severe than postoperative pain in terms of evaluation ( $p<0.01$ ). Differences between post insertion and postpartum pain failed to reach significance. Postpartum pain was rated as significantly more severe in terms of evaluation ( $p<0.001$ ) compared to postoperative pain.

A discriminant function analysis was carried out in the attempt to distinguish between the 5 pain groups on the basis of their score profiles. This classification method is designed to maximise the discrimination amongst groups defined a priori (15). The posterior probability of group membership (the probability of a patient belonging to each pain group conditional on their card sort scores) was determined from the discriminant functions and their prior probabilities (conditional only on the respective sample sizes) of group membership. According to these measures



Table V *Evaluations of individual cases based on proximity to closest group centroid*

Original pain group	Dysmenorrhea	IUD related pain	Post insertion pain	Postoperative pain	Postpartum pain
Dysmenorrhea	35	20	17	23	12
IUD related pain	2	19	4	7	6
Postinsertion pain	4	4	9	3	2
Postoperative pain	4	11	4	36	0
Postpartum pain	1	1	1	1	5

Figures shown are number of cases classified

every case was evaluated to determine how closely each patient's description of her pain as revealed by distribution of scores on the card sort resembled the average description by all patients with the same diagnosis. It was found that 42 per cent of all patients in the study resembled those of their own pain type more than any other and were thus correctly classified by the analysis (Table V).

Finally Pearson product moment correlations between factor scores and card sort totals were computed. The correlation matrix is shown in Table VI. It can be seen that all correlations are significant although not sufficiently high to indicate overlap and therefore duplicity.

## DISCUSSION

This paper presents a comparison in terms of both intensity and quality of common gynecology pains. The assessment method employed was found to have sufficiently high reliability and validity to justify such a detailed analysis (18). The multidimensional nature of this assessment allows the pattern of the pain response for each patient group to be described. This is felt to be important in terms of implications for pain management. For example Weisenberg (20) stressed the importance of psychological variables in the pain experience so that even where pharmacological

means are used to control pain the psychobiological status of the patient often determines their effectiveness. Beecher (4) has cited many instances of this for example the greater the stress the more effective the placebo. An analysis of the pain in terms of its various components may clarify treatment objectives. Thus the patient with low sensation and high evaluation may respond to reassurance and/or relaxation training without the need for analgesics.

Analysis of pain descriptions have been reported elsewhere (1, 5, 11, 12). Melzack and Dubuisson analysed the descriptions used according to 11 types (menstrual, toothache, cancer, arthritis, herpes, phantom, disc disease). They were able to correctly classify 76 per cent of the 93 patients in the sample to the correct pain group on the basis of questionnaire scores.

In the present study discriminant function analysis revealed a correct classification rate of less than 50 per cent. This was significant although not clinically useful in terms of predicting pain type from card sort scores. However the result should be seen in the context that the pain groups in this study were varieties of pelvic pain as compared with the Melzack and Dubuisson (6) study in which a wide range of pains were investigated. The widest difference in this study was between dysmenorrhea and postoperative

Table VI *Correlation coefficients between card sort total and factor scores*

	Sensory factor score	Tiring/temporal factor score	Evaluation factor score	Total
Sensory factor score		399	529	711
Tiring/temporal factor score			549	669
Evaluation factor score				884

All correlations significant at  $p < 0.01$  level

When these two were compared by discriminant analysis the correct classification rate increased to 70 per cent.

In terms of intensity comparisons dysmenorrhea received consistently higher scores than other pains. Interestingly while no difference in sensations was evident between dysmenorrhea and IUD related significant differences in terms of reaction to the similar levels of sensation did emerge. This corroborates the results of an early survey (16) in which no difference in sensation was found. It also conforms to predictions in that IUD relation pains may be better tolerated (give rise to less intensity) owing to the cause of the pain (the IUD) being clearly identified. Moreover women experiencing IUD related pain have chosen this method of contraception and the pain arising from the IUD can be offset against the positive benefits of contraceptive use.

The opposite is the case with dysmenorrhea as there are no positive aspects to diminish the significance of the pain.

Dysmenorrhea was rated as giving rise to significantly greater pain on all dimensions. This is also in accord with predictions as the postoperative pain which was related to a relatively minor gynecologic operation (laparoscopy) which had been elected by the patient and for which the prognoses were favorable. Sensations have been found to be better tolerated if the perceived cause has been voluntarily accepted by the sufferer as in the case of postoperative pain and IUD related pain (2).

While the degree of sensation did not differ between IUD related pain and post insertion pain the tolerance or reaction attached to these sensations was greater following the IUD insertion. Evidence suggests IUD insertion will be rated as less painful if patients are adequately prepared (17-14). This finds theoretical support from the present data as the reaction component was higher for post insertion pain stating the possibility that patients were insufficiently prepared for the sensations to be experienced. High levels of postpartum pain were reported in studies of both sensations and the reaction to these sensations. Once again it may have been expected that pain following delivery would have been well tolerated owing to its associations with a positive experience that of childbirth as the meaning of the situation in which the pain occurs has been shown to affect its intensity (3). Thus increased pain reports at delivery have been associated with ambivalent feelings about the prospect of motherhood (13). The pre-

sented results may have two implications. In the first place the high intensity of the pain sensations is noteworthy. Secondly the high reaction shown by patients in the present study may be the result of a lack of preparation for the possibility of such pain occurring. In the absence of such information the experience of pain may be associated with anxiety about possible complications and so be invested with undue significance. It would be predicted that inclusion of psychological preparation would have the effect of reducing the evaluative component of the pain.

In conclusion it appears that this method of pain assessment provides a meaningful way of describing gynecological pain. This survey has provided normative data on the severity of different pelvic pains in terms of quality as well as intensity and so permits comparisons of one with another. It also allows the clinician to compare individual patient scores with the average of the appropriate reference group. The profile of pain which emerges may have implications for management. For example both post insertion and post partum pain may be relieved by prior information and reassurance in order to reduce the significance that is attached to the sensations arising from each of these experiences. The profile of pain scores may also influence treatment of dysmenorrhea or pelvic pain of longstanding. In the course of running a pain clinic it has become evident that many patients are concerned by the significance of their pain experience which is reflected in high evaluative scores. It follows that substantial relief may be provided by focussing on the elevated reaction component of the pain and removing the worry surrounding the sensations.

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*Submitted for publication November 6 1978*

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# PHARMACOKINETIC STUDIES ON LOW DOSE ESTRADIOL 17 $\beta$ ADMINISTERED ORALLY TO POSTMENOPAUSAL WOMEN

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**Abstract** Peripheral plasma from four postmenopausal women was analysed for estrone estradiol luteinizing hormone and follicle stimulating hormone during 24 hours following an oral intake of a single dose of 1.0 mg micronized estradiol on the first day of therapy and after one month. A similar study was carried out with another four menopausal patients who received 0.2 mg estradiol three times daily. The measurements were performed by radioimmunoassay (RIA).

It was concluded that the plasma concentrations of estrone estradiol are higher and those of FSH lower after one month of therapy than on the first treatment day while LH remains unchanged. Micronized estradiol is well absorbed from the gastrointestinal tract and compared to estrone and the plasma profiles of estrone during therapy are more constant with a divided daily dose than with a single higher dose. The divided daily dose results in a usually good clinical effect even though the total administration of estradiol is lower.

Postmenopausal estrogen therapy for menopausal symptoms seems to be associated with an increased risk of endometrial cancer (5). Even though this risk may be reduced by giving an adequate cyclic progestin treatment with the estrogen therapy, an unnecessary high daily dose of estrogen is still a cause of concern. This makes it important to establish the lowest effective daily dose of estrogen. The aim of the present study was to determine the effect of reducing the daily amount of estrogen and dividing it into three doses.

The metabolism and pharmacokinetics of micronized estradiol were studied at the beginning of therapy and again after one month's treatment.

## MATERIAL AND METHODS

Healthy postmenopausal women participated in the study. The last menstrual period in each of these subjects was at least one year before the study but less than 10 years before the study. The exception had a menopausal age of 34-40 years before entering the study. Every subject had severe symptoms of vasomotor instability and had not used any hormonal medication for one month before the study. The results of the vaginal smears of each

subject were menopausal and atrophic. The breasts were studied by mammography and thermography and were considered to be normal. The subjects were randomly selected into two groups for treatment. The first group received tablets containing one milligram of micronized estradiol at 8 a.m. every day. The second group took a tablet containing 0.2 mg of micronized estradiol at 8 a.m., 11 p.m. and 8 p.m. The micronization of steroid and the quality of the tablet were the same. The tablets were specially prepared by Novo Industry A/S (Copenhagen, Denmark) for this trial. The absorption and effects of the drug were followed by collecting blood samples for the determination of plasma concentrations of estrone ( $E_1$ ), estradiol ( $E_2$ ), follicle stimulating hormone (FSH) and luteinizing hormone (LH) by radioimmunoassay (RIA). Blood samples were collected during the first day of treatment before the first intake and 1/2, 1, 1 1/2, 2, 3, 5, 7, 9, 12 and 24 hours after the ingestion of the tablet in the group receiving one milligram of  $E_2$ . In the second group the samples were collected on the morning before the intake of the tablet and then 1/2, 1, 2, 3 and 5 hours after the ingestion of each of the three tablets. Identical collections were performed after one month of treatment. The blood was collected into heparinized tubes, plasma was separated by centrifugation and stored at -20°C until analyzed. All samples were analyzed simultaneously.

$E_1$  and  $E_2$  were measured by RIA after diethylether extraction without chromatography as described by Edqvist & Johansson (3). The cross reaction of  $E_1$  in the  $E_2$  RIA was 1.24 per cent and that of  $E_2$  in the  $E_1$  RIA 1.65 per cent. The intra-assay coefficient of variation (CV) for  $E_1$  varied from 5.3 to 9.3 per cent and for  $E_2$  from 7.7 to 9.0 per cent. The interassay CV for  $E_1$  RIA varied from 16.0 to 18.9 per cent and for  $E_2$  from 9.3 to 10.5 per cent.

The RIA's of plasma FSH and LH were carried out as described earlier (7). The intra-assay CV was 9.3 and 2.4 per cent for FSH and LH respectively. The interassay CV was 14.7 per cent for FSH and 14.2 per cent for LH.

The evaluation of clinical effects involved comparing the frequencies of neurovegetative symptoms estimated subjectively by the patient before and after treatment (4, 9).

## RESULTS

The clinical effect of the treatment as evaluated by the patients was the same in both groups (Table I). There was one failure in both groups. The patient in the first group had persistently high gonadotropins.

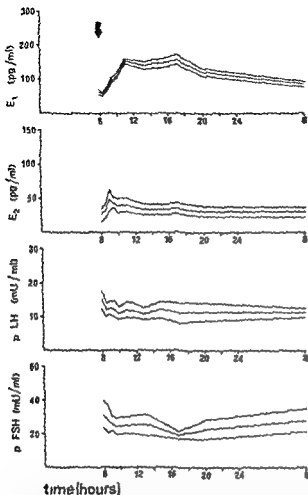


Fig 1 Plasma concentrations of  $E_1$ ,  $E_2$ , LH and FSH after ingestion of 10 mg micronized  $E_2$  at 8 a.m. mean and SE curves during one day. The arrow(s) indicate ingestion of the tablet(s).

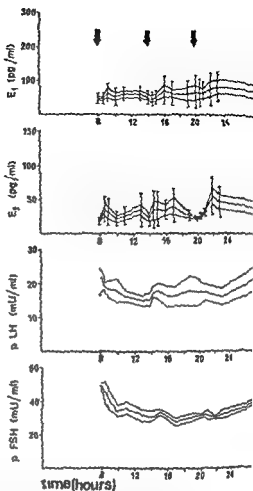


Fig 2 Plasma concentrations of  $E_1$ ,  $E_2$ , LH and FSH after ingestion of 0.2 mg micronized  $E_2$  at 8 a.m., 14 a.m. and 20 a.m. mean and SE curves during one day. For  $E_1$  SD also shown as vertical lines. The arrow(s) indicate ingestion of the tablet(s).

and the patient in the second group had no change in plasma  $E_2$ ,  $E_1$ , LH and FSH concentrations.

Table II gives the plasma concentrations of estrogens and gonadotropins at 8 a.m. before and after the treatment for one month. The basal values of estrogens were higher and gonadotropins lower in group I than in group II. After treatment for one month the mean morning levels of plasma estrone and estradiol were increased, the plasma concentrations of LH were unchanged but FSH decreased. The change during treatment expressed as a percentage of the pretreatment values was very similar in the two groups, except for plasma estradiol which was relatively more elevated in group II, where the treatment consisted of three doses a day.

The mean plasma concentrations of  $E_1$ ,  $E_2$  and FSH during the first 24 hours after the initial mg of  $E_2$  are presented in Fig 1. The plasma concentrations increased during the first three hours from 62 to 156 pg/ml and are clearly elevated 9 hours after the administration of  $E_2$ .

Plasma  $E_2$  reached a minor peak after one hour and tended to plateau five hours after the E. The LH plasma levels decreased during the first hours and remained at this level during the observation period. Plasma FSH concentrations first decreased rapidly, reached a nadir at the time of the estrone peak and increased slowly after that.

The average plasma concentrations of prog-

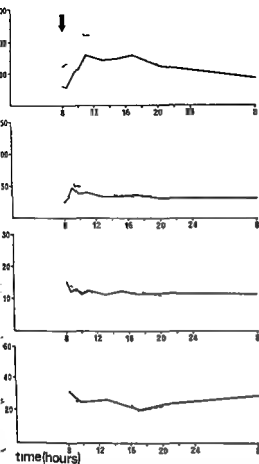


Fig 3 Plasma concentrations of  $E_1$ ,  $E_2$ , LH and FSH after ingestion of 1.0 mg micronized  $E_2$  at 8 a.m. Continuous line shows the mean plasma concentrations on the first treatment day (like in Fig 1) the broken line shows the mean plasma concentrations after one month's therapy. The arrows indicate ingestion of the tablet(s).

LH and FSH during the first day after three doses of 0.2 mg of estradiol are shown in Fig 2. Plasma concentrations of estrone increased slowly until the last sample on the first day. Small peaks one and three hours after the administration of the drug could be seen. Plasma estradiol increased slightly after each administration and a cumulative effect was seen at 10 p.m. when the mean level reached 55 pg/ml. Plasma LH was suppressed during the day but the 24-hour value reached the basal level. The suppression of plasma FSH was slightly stronger than that of LH.

The mean plasma concentrations of  $E_1$ ,  $E_2$  and LH for group I are given in Fig 3 and those of group II in Fig 4 for samples taken during the first

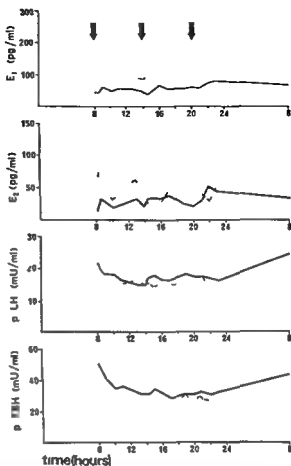


Fig 4 Plasma concentrations of  $E_1$ ,  $E_2$ , LH and FSH after ingestion of 0.2 mg micronized  $E_2$  at 8 a.m., 2 p.m. and 8 p.m. Continuous line shows the mean plasma concentrations on the first treatment day (like in Fig 2) the broken line shows the mean plasma concentrations after one month's therapy. The arrow(s) indicate ingestion of the tablet(s).

treatment day and the day after one month of treatment. The mean plasma concentrations of estrone in women taking 1 mg of  $E_2$  were higher after one month of treatment than during the first treatment day (Fig 3). The peak value was reached sooner but the plateau decreased at the same time as in the beginning of the trial i.e. nine hours after the ingestion of one milligram of  $E_2$ . The mean concentration curve of plasma  $E_2$  during the day after one month of treatment was very similar to that obtained on the first treatment day. The decreases in plasma LH and FSH resembled those seen during the first day.

As shown in Fig 4 the mean plasma estrone concentration was quite stable during the whole observation period. The mean level was however signifi-

Table I Clinical effect of the treatment. Patient's subjective evaluation after one month. Group I received 1.0 mg of  $E_2$  daily, group II 0.2 mg of  $E_2$  three times daily

Effect	Group I	Group II
Very good	2/4	2/4
Good	1/4	1/4
Poor	1/4	1/4

cantly higher after one month of treatment than during the first day and was about 100 pg/ml throughout the day. Plasma estradiol levels were also significantly higher after one month of therapy than during the first day of treatment. There were also peaks preceding the intake of the second and third doses. The gonadotropins were suppressed during the day time, but in group II the suppression was not as clear as at the beginning of the treatment, because the morning value of FSH was low.

### DISCUSSION

The results of the present study show that there are no significant differences in the morning plasma concentrations of  $E_1$ ,  $E_2$ , LH and FSH after one month of treatment when  $E_2$  is given as a single dose of 1.0 mg or as 0.6 mg divided into three doses. Therefore it seems that the daily dose of estradiol can be successfully reduced by dividing it into three doses. The divided administration also gave a more constant plasma level of  $E_1$  than a single daily dose, and the high concentrations associated with higher doses were eliminated.

The oral administration of estradiol resulted in higher plasma concentrations of  $E_1$  than  $E_2$  as shown before with micronized estradiol by Yen *et al* (10) and with estradiol valerate by Englund & Johansson (4) and Larsson Cohn *et al* (8). It seems that there is no difference in the relative plasma levels of

$E_1$  and  $E_2$  when the patients are treated with estradiol valerate or piperazine estrone sulphate (1).

The plasma concentrations of  $E_1$  and  $E_2$  were higher after one month of treatment than during the first day. This could reflect a saturation of the various pools. An alternative explanation could be that estradiol has a local effect on the intestinal mucosa which could increase absorption. There was a relatively higher increase in plasma estrone during the day of administration compared with estradiol after one month of treatment. This suggests an adaptation of the enzyme responsible for the conversion of estradiol to estrone. In this study vasomotor symptoms disappeared in all the patients who reacted to the treatment with elevated estrone levels and decreased gonadotropin concentrations. This is in sharp contrast with the results of Hämäläinen *et al* (6) who found no relation between the timing of flush and estrogen concentrations. However, this conclusion was based on fluctuations of low plasma concentrations of estrogens without any treatment.

A constant plasma concentration could perhaps be achieved with peroral medication by changing the micronization size of the tablets or even using sublingual capsules to avoid dividing the daily dose. This would be beneficial for the treatment.

In conclusion, rapidly absorbed micronized estradiol results in a good therapeutic effect by peroral administration and stable plasma concentrations are achieved even with a lower total dose by divided daily dose.

### ACKNOWLEDGEMENTS

This study was supported by Novo Industry A/S, Copenhagen, Denmark. The National Pituitary Agency, National Institute of Arthritis, Metabolism and Diseases is gratefully thanked for supplying the FSH and the antisera for these radioimmunoassays, also to Dr J. P. Raynaud for supplying the anti-steroid assays.

Table II Mean plasma concentrations  $\pm$ SEM of  $E_1$ ,  $E_2$ , FSH and LH at 8 a.m. in the two groups before and after one month of therapy with estradiol. Change from the pretreatment value is given in per cent.  $E_1$  pg/ml, LH and FSH IU/l

	Group I (n=4)			Group II (n=4)		
	before	after	change (%)	before	after	change (%)
$E_1$ (pg/ml)	63.1 $\pm$ 9.3	125.0 $\pm$ 20.0	+98.1	39.9 $\pm$ 4.3	84.0 $\pm$ 21.2	+110.5
$E_2$ (pg/ml)	26.3 $\pm$ 7.1	38.0 $\pm$ 10.0	+44.5	17.3 $\pm$ 3.4	31.5 $\pm$ 4.7	+82.1
LH (IU/l)	15.0 $\pm$ 1.6	14.4 $\pm$ 3.8	-4.0	21.9 $\pm$ 2.4	20.7 $\pm$ 2.6	-5.5
FSH (IU/l)	31.7 $\pm$ 5.1	24.1 $\pm$ 5.7	-24.0	46.8 $\pm$ 1.4	35.6 $\pm$ 1.8	-24.4

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*Submitted for publication November 8 1978*

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## ANNOUNCEMENT

**The Pan American Congress of Andrology** will take place in Mexico City Americana Fiesta Palace Hotel January 26–31 1981

*Information requests for registration and travel information should be directed to*

Dr A Negro-Vilar  
University of Texas Health Science  
Center at Dallas  
Southwestern Medical School  
Department of Physiology  
5323 Harry Hines Boulevard  
Dallas Texas 75235  
USA

*Information requests for registration and travel information should be directed to*

Gerald Bagatzinski  
Congress Administrator  
31600 West Chicago  
Livonia Michigan 48150  
USA

**The IIIrd World Congress of Human Reproduction** is to be held in West Berlin March 22–26 1981

### *Main topics*

- Central nervous system and regulation of reproduction
- Surgical and morphological aspects of reproduction in men and women
- Gonadotrophins and their target tissue
- Beginning of life *in vivo* and *in vitro*
- Experimental embryology of mammals
- High risk reproductive factors in breast and genital cancer
- Influence of the environment and drugs on reproduction
  - Free Communications
- Film Festival

### *Information*

Congress Secretariat  
Priv Doz Dr L Mettler  
Frauenklinik der Universität  
Hegewischstrasse 4  
D 2300 Kiel 1  
West Germany

**The Third International Congress on the Menopause** will be held in Belgium at the coastal resort of Ostend on 9–12 1981 just before the holiday season begins

This Congress will differ from the previous two (in Grande Motte 1976 and in Jerusalem 1978) in that as the aim in the past was to arrive at a consensus of opinion this time we shall bravely attempt to confront the controversies. It will however take the same form as the previous ones — a number of small workshops rather than one meeting attended by everyone

### *For further information*

The International Menopause Society  
8 av Don Bosco  
B 1150 Brussels  
Belgium  
Tel (02) 771 95 11 and (02) 771 96 45

**The Eighth Asian Congress of Obstetrics and Gynaecology** will be held in Melbourne in October 1981

An exciting and varied programme will be including expert plenary speakers from Asia and other Centres. The Congress themes include

- Population Control
  - Maternal and Perinatal Mortality
- Trophoblastic Disease
  - Gynaecological Malignancy
- Obstetrical and Gynaecological Endocrinology

The official language will be English

*Further information may be received from*

The Organizing Secretary  
VIIIth Asian Congress of  
Obstetrics and Gynaecology  
G P O Box 21957  
Melbourne 3001  
Victoria  
Australia

# DO ESTROGEN AND PROGESTERONE RECEPTORS ( $E_2R$ AND $PR$ ) IN METASTASIZING ENDOMETRIAL CANCERS PREDICT THE RESPONSE TO GESTAGEN THERAPY?

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**Abstract** In a prospective investigation biopsy specimens obtained of 150 women with primary untreated endometrial carcinoma for determination of the content of estrogen and progesterone receptors ( $E_2R$  and  $PR$ ). After a very interval 13 who developed metastases despite routine local and radiation therapy received treatment with gestagens. The clinical effect of the hormone was studied for correlation with the initial content of  $E_2R$  and  $PR$ . Eight of the women (61-71 years) proved  $E_2R$  positive (0 fmol/mg cytosolprotein). 2 had no  $PR$  and the remaining 6 had  $PR$  in a concentration of 7-892 fmol/mg cytosolprotein. 7 responded clinically to gestagen therapy (3 poorly differentiated in 3 and moderately in 4). Five of the women (50-84 years) were  $E_2R$  negative. They no  $PR$  they did not respond to gestagen therapy and died after 2-8 months (tumors poorly differentiated in 2 and moderately in 3).

Determination of  $E_2R$  and  $PR$  in endometrial carcinoma tumors may be a clinically useful test for predicting whether a tumor is likely to respond to treatment with gestagens.

Hormone therapy with progestagens can produce a significant regression in about one third of all cases of advanced or recurrent endometrial carcinoma. The average duration of the remission achieved by gestagen therapy alone in these cases of hormone-dependent adenocarcinomas is 14 months and the average duration of survival 2-3 years (21). The prognosis is usually best if the tumor is highly differentiated and the metastases are confined to the lungs. Response is also said to be better if the woman is young (15). However, excellent results have been obtained with low dosage progestagen therapy even in cases where the prognosis was initially very gloomy (1). Estrogenic vaginal smears may be another sign of tumor responsiveness to gestagen therapy while the level of estrone nor of estradiol in serum seems to be of any prognostic value (2). Progesterone seems to exert its effect locally on the cells of endometrial carcinoma leading to differentia-

tion, maturation and secretion and finally atrophy of the tumor epithelium. Evidence in support of this view has been produced by Nordqvist (18) and others.

It is claimed that the presence of specific cytoplasmic progesterone receptor protein ( $PR$ ) is necessary for progesterone to exert its cellular effect. Such receptors are generally demonstrable in normal endometrium but may also be found in endometrial carcinoma.  $PR$  activity is less if the tumor is poorly differentiated. Thus, in 23 cases examined Ehrlich *et al* (3) found a high  $PR$  content ( $>50$  fmol/mg cytosolprotein) in 85 per cent of highly differentiated cases, in 62.5 per cent of moderately differentiated ones, and in only 50 per cent of undifferentiated tumors. Irrespective of the degree of differentiation tumors that had previously been irradiated showed no evidence of  $PR$ . The  $PR$  content has also been found to vary inversely with the patient's age (22). The difference in  $PR$  content between highly and poorly differentiated endometrial cancer has also been demonstrated by Pollow *et al* (19) who in 15 cases examined found low concentrations of  $PR$  within a narrow range of 3-63 fmol/mg cytosolprotein. In a later series of 30 cases Pollow *et al* (2) found the highest  $E_2R$  content in those tumors that had a low  $PR$  content and that were undifferentiated. In contrast, in a recent study of 50 primarily untreated cases of endometrial carcinoma Friberg *et al* (6) found that  $E_2R$  was most often demonstrable in differentiated carcinoma, which also most often had receptors for androgen (DHT). Specific DHT receptors have been demonstrated recently in normal human endometrium (17). Of 11 patients with endometrial carcinoma Gustafsson *et al* found  $PR$  in nine, the content generally being lower in those cases where the tumor was poorly differentiated. Normal endometrium contained about 400 fmol/mg protein while endometrial carcinoma contained 0-62 fmol/mg.

Table I  $E_2R$  negative metastasizing endometrial cancers treated with gestagens Clinical data

Patient age	Tumor grade of differentiation	Metastatic spread to	Gestagen treatment and survival time
67	low	pulm	500 mg Proluton Depot 5 times weekly
		pelvic tissues	Progress 4 months
74	medium	omentum	500 mg Proluton Depot 5 times weekly
		pelvic tissues	Progress 8 months
77	medium	pulm	50 mg Depot Provera weekly Progress 3 months
50	medium	pulm	500 mg Proluton Depot 3 times weekly
		skeleton	Progress with neo pulm met 3 months
84	low	pulm	500 mg Proluton Depot 3 times weekly
			No effect but progress 2 months

The purpose of the present investigation was to find out whether the *in vivo* results of progesterone treatment of recurrences and metastases from endometrial carcinoma treated with surgery and radiation was correlated with the *in vitro* occurrence of  $E_2R$  and PR in the primary untreated tumor tissue

## MATERIAL AND METHODS

The material consisted of biopsy specimens of the endometrium obtained at diagnostic curettage of women with untreated primary endometrial carcinoma. Part of the tissue was fixed in formalin for routine histological examination and grading. Highly differentiated tumors showed glandular tubules with cylindrical epithelium while poorly differentiated tumors exhibited solid cellular areas or solid cords with epithelial cells. Moderately differentiated tumors showed a picture intermediate those of the other 2. Small pieces of tumors were also transferred to glass tubes immediately deep-frozen and stored until receptor analysis. Of the 150 patients from whom such specimens were obtained in the course of a 3 year period there were 13 who despite routine surgical and radiation treatment had a local recurrence or developed remote metastases which were then treated with gestagens. Pieces of the primary tumors from these 13 women were analysed for their content of estrogen ( $E_2R$ ) and progesterone (PR) receptors. The values found were studied for any correlation with the clinical effect of the hormone therapy.

The stored tissue was transferred into liquid nitrogen and vibrated in a fine powder by means of a microdismembrator. The powder was homogenized in cold buffer using a Ten Broeck homogenizer and centrifuged at 105 000 g for 60 min to prepare the cytosol. The cytosol was analysed for estradiol and progesterone receptor activity as published (1). In brief 50  $\mu$ l cytosol samples were transferred into the holes of microtiterplates and incubated with six different concentrations of  $^3H$   $E_2$  or  $^3H$  R 5020. The nonspecific binding for  $^3H$   $E_2$  and  $^3H$  R 5020 was determined in the presence of a large excess (1  $\mu$ M) of nonradioactive R 5070 and DES respectively.

After equilibrium was reached (16–20 hr at 4 °C) the bound steroid was removed by the addition of 10% dextran coated charcoal suspension. Scatchard plot analysis was performed to determine the concentration of receptor binding sites and the equilibrium dissociation constant ( $K_d$ ).

## RESULTS

To discriminate for the presence of estrogen receptors in our endometrial cancer patients values  $\geq 10$  fmole/mg protein were reported as positive. This cut off level for  $E_2R$  has previously been applied in the study of estrogen binding capacity of primary cancers. Clinical details of 5 such  $E_2R$  positive patients and the histopathology of their tumors are shown in Table I. None of the  $E_2R$  negative patients shown in Table I had any PR content in the primary tumor material analysed. In spite of gestagen treatment their survival was short, between 2–8 months without any certain relation to age or histopathology of the tumor.

Table II shows the clinical details and the histopathology of the tumors of 8 cases who were  $E_2R$  positive. With the exception of 2 cases they all had PR. 7 cases responded well to gestagen therapy with survival time of those who are dead of 6 to 18 and 20 months. 3 are still living symptom free.

## DISCUSSION

The possible importance of the presence of hormone receptors in endometrial carcinoma if gestagen therapy is to check spread of the tumor has so far been studied in only a few cases. The presence of receptors need not however mean that the tumor is responsive to hormone therapy since there may be a defect

Table II Data on age of patient histological grade of tumor differentiation amount of  $E_2R$  and PR protein) metastatic spread and the effect of gestagen treatment

Grade	$E_2R$	PR	Metastatic spread	Gestagen treatment and survival time
low	2	43	pelvic tissues ag na	1000 mg Depot Provera weekly Progress 3 months
low	133	89	skeleton	500 mg Proluton Depot 3 times weekly Improved Died of pulm embolia after 6 months
medium	37	7	supratrial and inguinal lymph nodes	500 mg Depot Provera weekly Improved with regression for 3 months then progress 11 months
medium	66	16	pulm	1 000 mg Depot Provera weekly Metastases rec essed for 9 months then progress 18 months
low	22	0	pulm axilla	1 000 mg Depot Provera weekly Regression for 8 months then progress 20 months
medium	70	72	pelvic tissue	500 mg Proluton Depot 3 times weekly After one year no residual tumor One year later symptom free
low	18	28	pelvic tissues	1 000 mg Depot Provera weekly Imp o After 2 years a first size pelvic tumor without symptoms.
medium	49	0	vagina	Proluton Depot 3 times weekly After 4 months no residual tumor One year later symptom free

Other factors necessary for a satisfactory hormone therapy (21) however reported 9 cases (with of 0-534 and PR of 0-2440 fmol/mg cytosol protein) including 4 that responded to treatment with progesterone 3 of these 4 had progesterone receptors. The fourth like the other 5 had no progesterone receptors. Of 12 cases of recurrent endometrial carcinoma Ehrlich *et al* (3) found a high PR content in 3 all of which responded objectively to progesterone therapy compared with only 1 of the 9 with a low PR content. They felt that an arbitrary value of 50 fmol bound  $H^3$  progesterone per mg cytosolprotein could be regarded as indicating a high PR content in the tumor.

In the management of breast cancer determination of both estrogen and progesterone receptors seems to increase the reliability of predicting whether any response to endocrine therapy can be expected in a case (11, 16). Mammary and endometrial tumors seem to have a similar endocrine background in many cases and therefore we include both  $E_2R$  and PR in our determinations. Our results show this to be of great importance from a predictive point of view and perhaps being the most decisive factor as in 2 of 10  $E_2R$  positive and responding cases no PR was demonstrable. This might however also be explained by the nature of the specimen if stroma was abundant and epithelial cells sparse. One case which was PR positive did not react to gestagens. If there had been any DHT receptors in this case it would have been interesting to try administration of an antiandrogen. Determinations of several receptors might increase their clinical value.

If carcinoma of the breast does not contain estrogen receptors it will practically never respond to endocrine therapy. But if such a tumor does contain receptors it will often respond favorably. The estrogen receptor level at which a response to treatment can be expected has been empirically found around 10 fmol/mg cytosolprotein. Instead of obviously meaningless endocrine therapy  $E_2R$  negative cases one can start aggressive cytostatic treatment without unnecessary delay. Borderline cases could receive combined hormone and cytostatic treatment. Cytostatics seem to be more effective when the tumor has no estrogen receptors (5, 13) which could imply a higher degree of malignancy in these cases. The absence of estrogen receptors in primary carcinomas of the breast is also believed to be a prognostically unfavorable factor and predicts early recurrence and early remote metastasis irrespective of such well known prognostic factors as the size of the tumor and the presence or absence of axillary metastases (4, 11). It appears that no attempts have been made to find out whether any association exists between the presence of receptors on one hand and the occurrence of metastasis to different locations such as the skin, vagina, lymph nodes, liver, lungs, brain etc. on the other. Owing to clonal differentiation the metastases might differ from one another and from the primary tumor with regard to the receptors. Metastases developing after treatment of the primary tumor might have different receptors from metastases arising before such treatment.

Progesterone appearing as an estrogen antagonist reduces the estrogen receptor content of both normal

and malignant endometrial cells (7) so that the cells are deprived of the stimulating effect of available estrogen. Synthesis of progesterone receptor protein however requires estrogen. If estrogen were given to a patient with endometrial carcinoma in a constantly low dose and progesterone were given intermittently in a high dose it might be possible to destroy neoplastic cells in non responding cases which initially lacked receptors (see also Jänne *et al*).

Adjuvant gestagen therapy might also be useful in the management of endometrial carcinoma in stage I. Administration of gestagen in such cases for a period of 2 years in a dose of 500–1 000 mg medroxyprogesterone per week plus conventional surgery and radiation appears to give an improved and almost 100 per cent five year survival rate (2). Since such a hormonal adjuvant has virtually no serious side effects objections of the type raised against adjuvant cytostatic therapy in mammary or ovarian cancer for example seem not to be applicable to progesterone. But even in such cases determination of the estrogen/progesterone receptors in the primary tumor tissue might be desirable in order to refine and adjust the treatment and the dose.

Measurement of the PR concentration in human endometrial tissues seems to be most accurately accomplished with R 5020 (1). This was the technique we used. It has to be stressed however that the tumor specimens were stored deep frozen for up to 3 years. It has been the experience of many authors and our own that the level of progesterone receptors in mammary carcinoma tissue may decrease strongly under such storage. This disturbing fact has to be taken into consideration when the levels of the progesterone receptor actually measured are regarded from the quantitative point of view. Therefore currently in our laboratory tumor specimens are determined without delay.

No definite correlation was found between E<sub>2</sub>R and the patient's age or the degree of differentiation of the tumors in our small study. This is of clinical importance if verified on a bigger number of patients.

Our findings show that *in vitro* method of determining E<sub>2</sub>R and PR can predict the *in vivo* response of patients with wide spread endometrial tumors to treatment with progesterone and that this is clinically useful. Cytostatic treatment can be given without delay to E<sub>2</sub>R/PR negative patients with tumors. Further research is needed to investigate whether the synthesis of absent E<sub>2</sub>R/PR receptors can be induced in tumor cells. It would be interesting to know whether

any positive correlation exists between treatment with estrogen in cases without E<sub>2</sub>R/PR content on the one hand and those with an increase in E<sub>2</sub>R/PR content on the other. This possibility is receiving attention in our laboratory and experiments to clear up this question are in progress.

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Submitted for publication August 26 1978

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1981

Date	Place	Name	Office
<b>1980</b>			
July 20-23	Williamsburg USA	AAGL Laparoscopic sterilization A comparison of the methods	American Association of Gynecol Laparoscopists 11239 South Lake Downey California 90241 USA
September 2-3	Barcelona Spain	Congreso Europeo Perinatal	VII Congreso Europeo de Medicina Perinatal Apartado de Correos 4 29015 Barcelona Spain
September 2-5	Barcelona Spain	7th European Congress of Perinatal Medicine	Congress secretariat Apt de Comer 29015 Barcelona Spain
September 2-6	Berlin Germany	6th International Congress of Psychosomatic Obstetrics and Gynecology	Ass Prof Dr M Stauber Frauenk Charlottenburg der FUB Pulsstr 4 D 1000 Berlin 19 W Germany
September 4-7	Kiawah Island Charleston SC	International Symposium on Carcinoma of the Cervix Biology Etiology & Diagnosis	E S E Hafez M D OB/GYN State University Medical Res Bldg 550 E Canfield Detroit MI 48201
September 15-16	Bologna Italy	First International Symposium on Recent Advances in Prenatal Diagnosis	A C Assistenza Congressi Via P Palagi 21-40138 Bologna Italy
September 22-28	Varna Bulgaria	3rd International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Aging	Bulgarian Academy of Sciences J G Vassileva Popova c/o Dept of Biophysics 113 Sofia Bulgaria
September 24-26	Kiel West Germany	International Symposium on Fertilization and Artificial Insemination	Priv Doz Dr L Mettler Frauenklinik der Universitat Kiel Hegewischstrasse 4 D 2300 Kiel
Sept Oct 29-1	Freiburg Germany	International Congress on Endocrinology of Human Infertility	C Ferrari M D P O Box 994 Milan Italy
September 30	London England	Anti androgen therapy for hirsutism	Symposium Secretary Inst of Obs Gynaecol Queen Charlotte's Hosp Hospital Goldhawk Road London W6 0XG England
October 3-5	New Delhi India	3rd International Seminar on Maternal and Perinatal Mortality Pregnancy Termination and Sterilization	Hon General Secretary The Fed Obstetric & Gynecological Society India Purandare Griha 31/c Dr N A Purandare Marg Bombay 400 007 India
November 14-17	Madrid Spain	7 Congrès Européen de Médecine Périnatale	Professor de Ill Fuente Maternidad de la Ciudad Av Generalissimo 177 Madrid 14
November 18-23	New Orleans Louisiana USA	AAGL Ninth Annual Meeting Clinical Symposium on gynecologic endoscopy	American Association of Gynecol Laparoscopists 11239 South Lake Boulevard Downey California 90241
November 20-22	Barcelona Spain	Symposium International Sobre Monitorización Prenatal	Instituto Dexeus Srtas M Luis Ave Ana Baldrich c/Paseo de la Br 67 Barcelona 17 Spain

## INTRAEPITHELIAL NEOPLASIA AND CARCINOMA IN THE VAGINAL VAULT

Helen Marie Henriksen

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An 11 year biopsy material from the vagina is studied. The indication for taking the 370 vaginal biopsies was normal cytology, polyps or suspicion of tumor. The material was found to include 10 cases of intraepithelial neoplasia, viz. 4 primary, 6 secondary, and 2 cases of secondary invasive carcinoma. The interval between the primary and secondary diagnosis ranged from 7 months to 19 years. It is concluded that the risk of secondary changes in the vaginal mucosa is slight, but cytological follow up is recommended.

Primary invasive carcinoma of the vagina was first described by Cruveilhier (3) in 1827 in an autopsy material. In 1911 authors (1, 21) have reported that its share in the malignancies of the female genitalia amounts to 1 per cent or 3.6 per cent. Carcinoma *in situ* (CIS) of the vagina is primary or secondary to neoplastic changes in the cervix. It is stated to be even more rare (5). Intraepithelial vaginal carcinoma is diagnosed at the intraepithelial stage; the survival time is the same as for CIS of the cervix (18). Although intraepithelial changes in the vagina develop but slowly into invasive carcinoma, progression to malignancy is on record (9, 13, 22).

Since this development has taken place, the prognosis in terms of 5 year survival is considerably improved. In 1952 Graham & Meigs (6) reported recurrence in 10 patients with a history of hysterectomy and intraepithelial neoplasia of the cervix. This altered surgical technique, so that hysterectomy for cervical CIS was made to include removal of the vaginal vault, with thorough histological examination of the vaginal margins and a more frequent cytological control of the remaining vaginal vault.

The object of the present paper is to survey the frequency and treatment of invasive and intraepithelial neoplasia of the vaginal vault from an 11 year biopsy material in the Gynecological Department and Pathology Department of Frederiksberg Hospital, Copenhagen.

## MATERIAL

A retrospective review of 370 biopsies from the vagina taken in the Gynecological Department during the period 1.1.1966 to 1.1.1977 on the indication abnormal cytology, polyps or suspicion of tumor tissue disclosed 10 cases of intraepithelial neoplasia in the vagina, primary or secondary, and 2 cases of squamous-cell carcinoma. The biopsies and the corresponding clinical material will be presented below.

## RESULTS

Table I lists the patients' ages, the histological or cytological diagnosis on which primary and secondary treatment was based, mode of treatment and length of follow up period.

Four of the 12 cases were primary and 8 secondary, having previously had intraepithelial neoplasia or carcinoma of the cervix. Of the 4 primary cases, two patients had a history of hysterectomy for benign disease (Cases 11 and 12). Positive cytology in subsequent cancer screening indicated biopsy, which disclosed vaginal dysplasia and CIS respectively. Repeated findings of suspicious cells (Cases 9 and 10) indicated cervical curettage and biopsies from the portio and vaginal vault. In one of these patients (Case 9) conization was done because of dysplasia in the cervical biopsies. Histological examination of the conus showed no abnormalities, but there was severe dysplasia in the vaginal vault. The other patient (Case 10) exhibited no abnormalities in the cervical smears or biopsies from the portio, but mild dysplasia in the vaginal vault. Of the 8 secondary cases, five (Cases 2, 3, 4, 5 and 8) had previously had cervical CIS, one (Case 1) dysplasia, and two (Cases 6 and 7) Stage I squamous-cell carcinoma.

The primary treatment had been hysterectomy in 7 patients (Cases 1, 2, 3, 4, 5, 6 and 8) and radium in one (Case 7). The interval between primary treatment and the finding of secondary histological changes of the vaginal vault requiring treatment ranged from 7 months to 19 years.



Table I Age diagnosis and treatment (prim and sec) and period of observation after sec treatment

Pat no	Age and diagnosis at prim treatment	Prim treatment	Age and diagnosis at sec treatment	Sec treatment	period of observation
1	56 dysplasia	hysterectomy	57 dysplasia	radium	6 yr
2	49 ca in situ	hysterectomy	54 dysplasia	0	3
3	35 ca in situ	hysterectomy	36 dysplasia	0	3
4	59 ca in situ	hysterectomy	60 abn cytology	radium	9 yr
5	38 ca in situ	hysterectomy	53 c plano-cellulare	radium + ext radiation	11 yr
6	46 stage Ia	hysterectomy	49 ca in situ	radium + ext radiation	9 yr
7	45 stage Ia	radium	64 ca in situ	hysterectomy + ext radiation	1 yr
8	42 ca in situ	hysterectomy	54 c planocellulare	radium + ext radiation	10 yr
9	54 abn cytology	conization	dysplasia	0	4
10	45 ca in situ	0	dysplasia	0	2 yr
11	47 benign disease	hysterectomy	dysplasia	0	4 yr
12	56 benign disease	hysterectomy	ca in situ	radium	9 yr

repeated

**Secondary treatment** Three (Cases 9, 10 and 11) of the four patients with primary intraepithelial changes in the form of dysplasia received no treatment as repeated cytological studies showed no abnormalities. One patient (Case 12) with CIS was treated with radium. The follow up period is from 2–9 years and no patient has shown signs of recurrence.

Out of the 8 secondary cases two patients (Cases 2 and 3) with dysplasia in the vaginal vault received no treatment as the cytological findings were negative during the next 3 years. One patient with dysplasia (Case 1) and persisting positive cytology was treated with radium. Four patients (Cases 4, 5, 6 and 8) one of whom had repeated abnormal cytological findings, two squamous cell carcinoma and one CIS of the vaginal vault, were treated secondarily with radium. The last 3 (Cases 5, 6 and 8) also with external roentgen radiation. Six of these 8 patients had been free of recurrence during a follow up period of 3–11 years while one (Case 8) developed in the course of a few months a vesicovaginal fistula and recurrence of the tumour in the entire vaginal wall and on the labia majora. Finally Case 7 who had been treated 19 years previously with radium for cervical carcinoma underwent hysterectomy. The operation was not radical and one year later it was supplemented with external roentgen radiation because of tumour tissue in the vaginal vault.

### DISCUSSION

Theories concerning the pathophysiology of neoplasia in the vagina have been advanced by Palmer &

Spratt (16) among others. They believed condition could be induced by irradiation of vagina in low doses which had a stimulating effect upon the growth of abnormal cells in the vagina. One of the present patients had previously been treated by radium. Newmann & Cromer (17) and others (2, 4, 11, 14) later suggested the theory of multicentric origin of carcinoma in the vagina and vulva. They believed that the tumour arose from multiple mutually independent foci rather than as a result of a common carcinogenic stimulus. Workers (8, 9, 18) have demonstrated carcinoma *in situ* in the vulva as well as in the vagina of the same patient. Only one of the patients in the present material had changes in the vulva (8).

Table I shows a difference of up to 19 years (7 years) in the patients' ages at primary diagnosis. It is a natural explanation that development of intraepithelial changes is very slow and possibly starts later in the vagina than in the cervix. However, changes of the vaginal mucosa have been present at the primary treatment but escaped diagnosis. Similar intervals in age have been reported by others (5, 9, 10).

The primary as well as the secondary treatment was carried out in the Gynecological Department. The primary cases were diagnosed in the course of the cancer screening programme in the municipality of Frederiksberg and the 8 patients with secondary changes had been followed in the Outpatient Clinic for 5 years after primary diagnosis and treatment.

most common abnormal but guiding finding positive cytology. No patient had vaginal discharge, spotting or post coital bleeding. If the portio has been preserved, a positive smear may lead to the diagnosis and treatment of a possible cervical disease, reducing the risk that dysplasia or CIS of the vaginal vault is overlooked.

In posthysterectomy patients: positive cytology should always be followed by staining of the vaginal vault with Schiller's solution, followed by biopsy. According to Schiller (19), the test is based upon the fact that normal squamous epithelium is high in glycogen, stains brown with a solution of iodine, whereas epithelium with intraepithelial changes or carcinoma is devoid of glycogen and therefore remains uncoloured. Experience has shown, however, that certain benign diseases (hyperkeratosis, erosion, metaplasia) remain unstained and that conversely mild to moderate dysplasia and even carcinoma *in situ* or invasive carcinoma may stain with a solution containing iodine. However, although the Schiller test may give a false positive result in benign diseases and a negative result in malignancy (17), it is still being used because of its simplicity by many gynaecologists to demarcate mucosal areas to be biopsied or excised. Colposcopy of the vaginal vault may possibly be used.

Atrophic mucosae in postmenopausal women should be treated with estrogen prior to the Schiller test and biopsy, since atrophic epithelium usually does not contain the quantities of glycogen needed for iodine staining. Invasive vaginal cancer following hysterectomy for carcinoma *in situ* is rare. The present material includes two cases of squamous-cell carcinoma in the vagina, diagnosed 15 and 12 years after primary treatment.

The interval of many years between the demonstration of mucosal changes on the portio and in the vagina (5-9, 18) supports the theory of a multicentric origin. On the other hand, a recurrence within the first 3 years after primary treatment in 4 patients may be explained by the operation not having been radical, leaving precancerous changes in the vaginal vault. Thus, revision of the microscopic preparations showed dysplasia in the surgical margins in 3 of the 4 cases, but no abnormality in the fourth.

Cases indicating removal of the uterus because of intraepithelial neoplasia in the cervix: most authors (1, 2, 12) agree that the uppermost centimetre of the vagina should be removed as well. Empirically, the rare recurrences are nearly always situated in

the vaginal vault, and indeed this was so in 7 out of the 8 patients in the present material, despite removal of the vaginal vault at the time of hysterectomy. Only one patient (Case 8) had malignant changes in the entire vagina as well as in the vulva.

When treating intraepithelial neoplasia of the vaginal vault, a few workers (7, 9) prefer local excision instead of radical hysterectomy in order to avoid its side effects. Five of the present patients received no secondary treatment because of subsequently negative cytology. The changes observed had presumably been removed by the biopsy forceps.

## CONCLUSION

Early diagnosis of a recurrence of intraepithelial neoplasia in the vagina is important. As there is a possibility of subsequent invasive carcinoma, cytological study should probably not be omitted in patients with a history of hysterectomy for benign disease, although in such cases there is little risk of intraepithelial and invasive carcinoma of the vagina. Total hysterectomy for CIS or dysplasia should include the uppermost centimetre of the vagina. In spite of a minimal risk of recurrence, continued follow-up of these patients by smear tests is advisable, especially as they often do not have symptoms. It is recommended to continue the follow-up for many years, perhaps for life. In the event of positive cytology, the vagina should be stained with iodine, possibly after preceding estrogen therapy, and biopsies taken from non-staining areas. Colposcopy may afford guidance.

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*Submitted for publication July 27 1978*

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## EFFECT OF ESTROGEN/PROGESTOGEN COMBINATIONS ON POLYMORPHONUCLEAR LEUCOCYTE CHEMOTAXIS

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**Abstract** The effects on leucocyte chemotaxis and on the lymphocyte culture reaction were studied in fourteen women taking estrogen/progestogen combinations. An inhibition of leucocyte chemotaxis was found during hormonal treatment and sera from these women were found to inhibit lymphocyte culture reaction.

Combined estrogen/progestogen preparations are used by numerous healthy women over prolonged periods for hormonal contraception or substitution during the menopause. Recent data suggest that despite the variety of clinical effects following such hormone therapy, some may reflect interference with immune mechanisms (12-21). Estrogen seems to have an inhibitory effect on the thymus (15-19) and in laboratory animals the administration of estrogen/progestogen has prolonged the survival of homozygous (14-15). During human pregnancy a depressed activity of the maternal T lymphocytes has been demonstrated in several investigations (18-23). It has been shown that plasma or serum from pregnant women has immunosuppressive properties (5-13, 24). Similar reactions have been observed in women using oral contraceptives (1-2, 9) but the results are conflicting (6). Women taking oral contraceptives have been reported to have an increased risk of developing chicken pox (varicella) and a decreased risk of non-toxic goitre, thyreotoxicosis, myxedema, rheumatoid arthritis. Lymphomononuclear leucocyte (PMN) function is an important part of the immune response and an increased phagocytic and bactericidal activity during pregnancy has been reported (16). So far we have found no reports concerning neutrophil leucocyte function during estrogen/progestogen treatment. In the present investigation neutrophil chemotaxis was studied in women during such treatment and the effect of serum on control leucocytes and the mixed lymphocyte reaction were studied.

## MATERIAL AND METHODS

**Subjects** Fourteen women were studied before and after 2 months of treatment with estrogen/progestogen preparations. Eight women, age range 41-56 years, were treated with a combination of estradiol valerate 2 mg/norgestrel 0.5 mg (Cyllabul<sup>®</sup>). Ten women, age range 16-23 years, were treated with etynylestradiol 0.03 mg/norgestrel 0.15 mg (Follimin<sup>®</sup>, Neovietta<sup>®</sup>). All women were apparently healthy, taking no other drugs, and there were no clinical signs of infections or other diseases during the observation period.

**Chemotactic assay** Leucocyte chemotaxis was studied in modified Boyden chambers by the leading front technique of Wilkinson (25). Ten ml of venous blood samples were allowed to sediment in plastic tubes for 30 minutes at 37 °C in the presence of 2 ml 1 per cent metosel solution. The leucocyte rich supernatant was diluted in Gey's solution pH 7.2 (25) and centrifuged at 500 × g for 5 minutes. The cells were washed 3 times and finally resuspended in Gey's solution to a final concentration of  $1 \times 10^6$  cells per ml. The cell suspension (0.2 ml) was placed in the upper part of the chemotactic chamber. In the lower part was placed a) 3.6 ml of Gey's solution as negative control or b) 3.6 ml of casein solution (5 mg casein per ml Gey's solution) (6). The chambers were incubated at 37 °C for 20 minutes and the filters (porosity 3 microns/Sartorius Inc., Göttingen, Germany) were then removed, fixed and stained (6).

The filters were inspected at the magnification of ×400 and the distance travelled by the leading front leucocytes was measured as the mean of 5 different fields. Each experiment was duplicated. In this system chemotaxis of controls ranges 100 to 130 microns. The mean values ± SD of chemotaxis for seven healthy control donors before and after the investigation period were  $121.5 \pm 21.7$  and  $114.9 \pm 12.7$  microns respectively.

**Incubation of control leucocytes** To evaluate separately cellular and humoral factors, serum samples from the treated women were used for incubation of leucocytes from healthy untreated donors prepared as described above. Incubation with patients' sera was allowed for 1, 2 and 24 hours at 37 °C. Chemotaxis of control cells was then performed as described.

**Mixed lymphocyte culture** Venous blood from unrelated healthy nonpregnant donors was defibrinated by gentle agitation with glass beads. The lymphocytes were separated by centrifugation on a layer of Ficoll Isopaque (4). The cells were washed 3 times and suspended in medium RPMI 1640 containing 20 mmol/l Hepes (Flow Laboratories). Strepto-

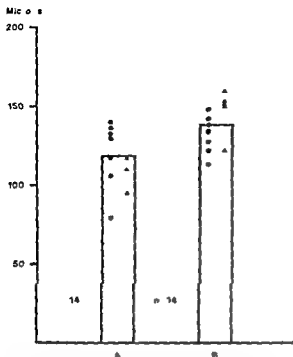


Fig 1 Neutrophil chemotaxis before (A) and after (B) hormonal treatment. Filled circles represent women taking estrogen/progestogen for climacteric disorders; filled triangles women taking oral contraceptives.

mycinsulphate was added in 100 µg per ml of medium. Wells of Falcon Microtest II tissue culture plates (Gateway International, Los Angeles) were filled with 150 µl of mixed lymphocyte suspension with equal numbers of lymphocytes from each of two donors. To each well 50 µl of serum from one of the investigated women or autologous serum was added. The final concentration of lymphocytes was  $1.5 \times 10^6$  per ml. All tests were done in triplicate. The microplates were closed with a plastic film and incubated at 37 °C for 6 days. Five hours before the end of the incubation period 0.04 µCi of [ $^{14}$ C] thymidine in 20 µl saline was added to each culture. Cultures were washed and precipitated on glass fibre filters by a semiautomatic multiple sample processor and the radioactivity was determined in a scintillation counter. Quenching estimated by the external standard method was very similar throughout each experiment and therefore results were expressed as the average of the counts per minute obtained in triplicate tests.

## RESULTS

Neutrophil chemotaxis before and after hormonal treatment is shown in Fig 1. The migration increased from  $118 \pm 7.4$  microns (mean  $\pm$  SD) to  $137.9 \pm 13.7$  microns. Using Student's *t* test for paired observations the increase was highly statistically significant for the whole group ( $p < 0.001$ ) and for the subgroup

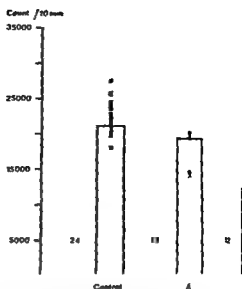


Fig 2 Comparison of the mixed lymphocyte reaction between control cultures containing autologous sera (A) and after hormonal treatment (B). Filled circles represent women taking oral contraceptives; filled triangles women taking oral contraceptives.

of six women taking 0.03 mg ethinylestradiol/norgestrel ( $p < 0.001$ ). The random migration was  $23.3 \pm 3.4$  microns (mean  $\pm$  SD) before and  $23.3 \pm 3.4$  microns after 2 months of treatment. Control donor leukocytes with patient sera after 2 months of treatment gave no changes in migration for any of the three periods used.

The influence of patients' sera on the mixed lymphocyte reaction using lymphocytes prepared from unrelated control donors is shown in Fig 1. There was no difference between control cultures containing autologous serum and cultures containing sera before hormonal treatment. A significant increase of lymphocyte reactivity was found in cultures incubated with sera from women after 2 months of hormonal treatment. This was true for the whole group ( $p < 0.01$ ) and for the subgroup of patients taking estradiol valerate 2 mg/norgestrel (Clylabil®) ( $p < 0.05$ ).

## DISCUSSION

Chemotaxis, adherence, phagocytosis and cellular killing of microbes are fundamental functions of polymorphonuclear neutrophil leucocytes.

ical conditions with various defects in this system an increased susceptibility to infectious diseases have been described (20). A decrease in neutrophil chemotaxis is known to occur during treatment with the antibiotics (21). So far we have found no data concerning this aspect of host defence during combined estrogen/progestogen treatment in women and moreover few factors are known to increase chemotaxis. Ascorbic acid (8) and certain infections have been reported to increase chemotaxis as well as the spontaneous random migration. Recently small doses of alcohol were found to increase neutrophil chemotaxis in an in vitro experiment (10). During pregnancy the neutrophil phagocytic function has been reported to increase (16). The increased chemotaxis found in this study seems to be due to other factors since incubation with serum did not show any change in the migration capacity of control lymphocytes. Neutrophil chemotaxis is influenced by lymphocytes since lymphokines have been shown to depress and to increase chemotaxis (22). During pregnancy there is a depressed T lymphocyte function and a few reports have described a similar phenomenon in women taking oral contraceptives (1). In the present study sera from women during hormonal treatment gave an inhibition of the MLC reaction from unrelated control lymphocytes. It is established that pregnancy plasma has the same properties. It might be that depression of lymphocyte activity during hormonal treatment may reduce lymphocyte activity resulting in an increased neutrophil chemotaxis. An increase of neutrophil chemotaxis and an inhibition of T lymphocyte activity might represent an altered immune response during combined estrogen/progestogen treatment. Further studies are necessary to clarify whether these observations have any relation to the increased susceptibility to infections in viral infections and autoimmune diseases reported in women undergoing such therapy.

#### ACKNOWLEDGEMENT

This investigation was supported by grants from the Swedish Medical Research Council (project No. 4217) from the Medical Faculty, University of Umeå. The Tore Nilsen Foundation for Medical Research, Jubileumsklinikens Stiftelse, Stiftelsen Allmänna Barnbördshuset and Skilfuld Technical assistance was provided by Agneta Andersson, Mrs Monica Isaksson and Mrs Birgitta Wallen.

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Submitted for publication September 1 1978

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# ULTRASTRUCTURE OF CELL DETACHMENT FROM THE HUMAN FETUS IN EARLY PREGNANCY

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**Abstract** The recently confirmed value of amniotic fluid cytology as a tool in the determination of fetal maturity constitutes the background of the present study in which the exfoliative capacity of human fetal skin was investigated by correlating ultrastructural methods. By transmission and scanning electron microscopy detachment mechanisms are described in the fetal periderm. It is concluded that the exfoliative capacity prevailing in early pregnancy fetal skin is different from the maturity related exfoliative capacity in third trimester. Both kinds of exfoliated cell material constitute antenatally available fetal tissue: the utilisation of both in antenatal diagnosis may be extended.

The origin of cells in the amniotic fluid has recently been investigated in the Rhesus monkey and in the human in early and late pregnancy (1, 2, 3). These studies are concerned mainly with the cell pattern in the amniotic fluid as an expression of the tissue differentiation in the fetus. Using cytology tissue maturity can be determined with high accuracy in particular in risk cases complicated by meconium contamination of the amniotic fluid where biochemical assessment of phospholipids is unreliable (7, 8).

In early pregnancy cell morphology of the amniotic fluid has been studied only to a small extent due to limited information obtainable from these cells supported by chromosome analysis. In the light of recent findings in late pregnancy concerning maturity determination by cytology however a growing interest is evident regarding the contribution in early pregnancy of various tissue surfaces to the cell contents of amniotic fluid. A striking difference in the degree of detachment of cells to the fluid has been shown mainly the amniotic epithelium virtually lacking exfoliative capacity while fetal periderm displays heavy desquamation (2). The nature of this phenomenon is unclear as is its occurrence in some ectodermic vesicles but not in others.

The cells and cell fragments detached to the amniotic fluid may constitute messengers emitted from the fetus in early pregnancy. These tissue fragments are unique because of the ease by which they are obtained as the first tissue samples available from the fetus *in utero*. In a future widening of the field of antenatal diagnosis the understanding of the site of exfoliation and the nature of cell detachment from certain epithelia will probably elucidate the role of these cells and what they express. The purpose of the present study was to correlate parallel transmission (TEM) and scanning electron microscope (SEM) findings on the formation of cells and cell fragments from the fetal periderm.

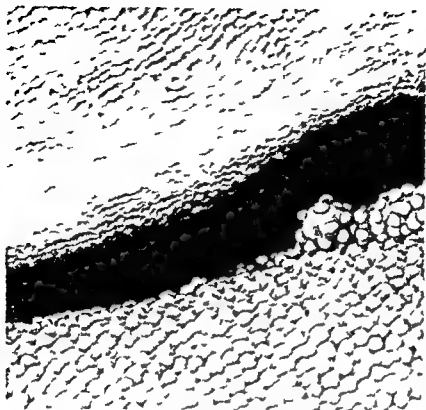
## MATERIAL AND METHODS

Tissue material was harvested from 10 pregnant women in 16th to 19th week undergoing hysterotomy for legal abortion. The amniotic sac was evacuated and torn in 2.5 per cent glutaraldehyde in Soerensen's phosphate buffer pH 7.4. After immersion in the fixative for at least a few days the material was dissected and specimens of skin from the thigh and the back were excised in small portions for further preparation. After rinsing specimens in Soerensen's buffer they were postfixed in 1 per cent osmium tetroxide in the same buffer.

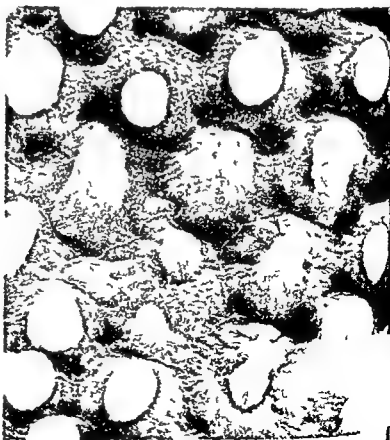
For TEM specimens were processed in a dehydration series to absolute ethanol and then gradually to propylene oxide and Epon. Sections for light microscopy were taken and toluidine blue stained and representative areas selected for fine sectioning on an LKB ultratome. The fine sections were stained with uranyl acetate and lead citrate. A Philips 300 A transmission electron microscope was utilized.

For SEM specimens were taken through three baths of redistilled water frozen with a minimum amount of water by plunging the tissue pieces in a quenching medium of isopentane chilled by surrounding liquid nitrogen. Freeze drying was carried out at about -17°C (dry ice) for 2-3 days. After mounting on SEM stubs sputter coating with gold was carried out. A JEOL JSM U3 scanning electron microscope was used run at 15-20 kV.

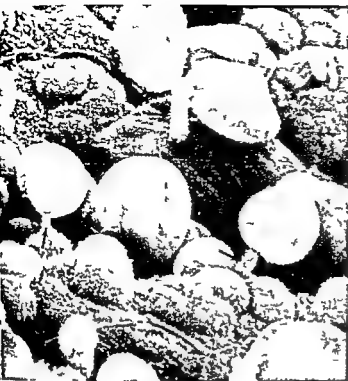




*Fig 1* Survey over fetal week 19. Note bulging cells instances partially the peridermal surface electron microscopy



*Fig 2* Cell fragments in various stages of exfoliation from human periderm 19th week. Delicate strands of intercellular borders constitute a polygonal pattern of fetal skin cells. In the center of each cell a protrusion is formed densely microvillous and gradually undermined by several indentations (arrows). Scanning electron microscopy magn 1700 x



*Fig 3 Detachment of cell fragments from polygonal cells of fetal skin (periderm). Note visible stalks (arrows) carrying two structures apparently liberated from the periderm to the amniotic fluid. The microvillous surface and the different stages of exfoliation are visible. Scanning electron microscopy magn 2 500  $\times$*



*Fig 4 Transverse section through fetal periderm 37 weeks showing skin surface uppermost with cell fragments in the course of exfoliation. Two indentations (arrows) near the base of protruding cell fragment forming microvillous cavities (cf Fig 6). The most superficial cell layer is the fetal periderm (P) while underneath the germinative layer is distinctly multilayered and contains an abundance of glycogen granules (G). Transmission electron microscopy magn 2 500  $\times$*



Fig 5 Glycogen fragments under exfoliation from fetal periderm in amniotic fluid. No cell surface and glycogen granules (G). The germinal layer (GL) and periderm are attached to each other by a system of desmosomes (Fig 7). Transmission microscopy magn 103,000.

## RESULTS

Surface ultrastructure of the skin specimens investigated showed features of extensive detachment of cells and cell fragments. Both in TEM and in SEM there was a striking uniformity of the exfoliation procedure. Several distinct stages of detachment could be detected and confirmed by means of both techniques. The earliest sign of a forthcoming exfoliation was the occurrence of a dense microvillous lining on a convex cell surface. This lining was typically more dense towards the center of the microvillous area (Figs 1–2). Gradually the center was slightly elevated to form a rounded, densely microvillous prominence. In this slight elevation small pits and minor invaginations were obvious in the lowermost portion (Figs 3–4). By and large these pits coalesced to form confluent spaces below the continuously more bulging prominence (Fig 5). In the periphery of the protrusion an invagination took place and brought about further undermining of the protrusion. The confluent spaces below the protrusion showed a microvillous lining surrounding a secretion mostly amorphous but sometimes granular (Fig 6).

Exfoliation to the amniotic fluid always occurred from the outer layer (periderm) while the second layer (germinative layer) never showed any signs of cell surface detachment. Between these layers an extensive network of desmosomes was conspicuous (Fig

7). All along the thin unicellular periderm there were desmosomal connections to the germinative layer underneath. In both layers a prominent feature was the occurrence of an abundance of glycogen granules, either as single particles or as rosettes (Fig 8). There was a gradual loss of glycogen granules in the deeper parts of the germinative layer.

Tonofilaments were predominant in the peridermal cytoplasm (Figs 9–10) and were particularly concentrated around the cell membrane indentations and coalescing pits. The filaments were closely interwoven with areas of glycogen granules. The undermining of elevated globular cell portions brought about tonofilament-rich areas of the peridermal cytoplasm, which seemed to progress to a continuous decrease in the cytoplasmic communication between exfoliating cell fragment (or cell) and the peridermal network. The end result, as illustrated by SEM, could be confirmed by TEM as protruding pedicles of cytoplasm, seemingly remnants of cytoplasmic bridges from periderm to the cytoplasmic portion recently pulled off in the exfoliation process.

## DISCUSSION

The outer surface of peridermal cells is endowed with a capacity to create membrane protrusions and to increase the available periderm area during



Transversely cut indentation undermining exfoliating periderm segment to the amniotic fluid. The microvilli present the same appearance as on the surface of the detaching periderm fragments. Note finely granular ultrastructure of the periderm present in the indentation. Transmission electron microscopy magn 90 000  $\times$ .



Fig 7 Attachment zone between periderm (P) and germinative layer (GL) of human fetal skin 19th week. Note distinct desmosomes (D) connecting the two layers and also an apparent direct communication between the two cytoplasms (arrow). Transmission electron microscopy magn 90 000  $\times$ .

ation of the epidermis *in utero* this capacity is used only during a rather limited period of gestation, ranging from around the fourth to the sixth month of pregnancy (5). Prior to the sixth month the epidermis is built up with a multilayered germinative part and a superficial monolayer, the periderm. From the seventh month onwards a permanent cornium is established and the periderm is subjected to regression (4). During the active period of regression the fetal periderm is strikingly different from the other important component of the lining of the amniotic cavity, the amniotic epithelium, which strikingly lacks the capacity of membrane motility characteristic of fetal periderm (2, 1).

The exfoliative behaviour of fetal periderm runs concomitantly with various membrane events leading to a vast plasma membrane area exposed to the amniotic fluid. These events suggest that fetal periderm is not only a protective layer shielding the fetus but

also an active participant in transport phenomena between fetus and amniotic fluid. The occurrence of cytoplasmic vesicles subjacent to the outer peridermal plasma membrane would support the suggestion of exchange across peridermal cells (4).

Membrane motility in the cell area facing the amniotic fluid but not in the area facing the germinative layer could be explained by the conspicuous network of desmosomes connecting the peridermal monolayer to the germinative cells underneath. The occurrence of an abundance of tonofilaments in peridermal cells, particularly around indentations and coalescing pits, suggests that these filaments are somehow related to membrane motility. This suggestion seems validated by the close interrelationship between filaments and glycogen granules as evidenced in the present study. Energy built up in animal cells in the form of carbohydrates is usually stored as glycogen. Most cells in the periderm and the germinative layers were virtually

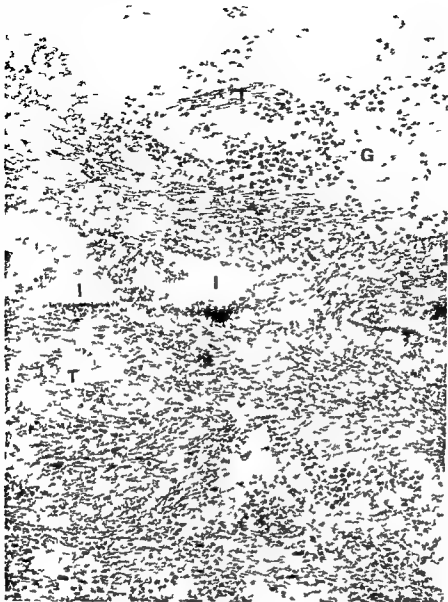


Fig 8 Glycogen granules are often intermingled with tonofilaments (T) in the uppermost part of the fetal epidermis. In the lower part a more scattered arrangement of single glycogen granules and dispersed tonofilaments is evident. A large indentation (I) is the middle lamella. Transmission electron micrograph 60 000  $\times$

stuffed with glycogen granules. The origin of these granules is not known but an exchange between peridermal glycogen and amniotic fluid glucose has been suggested (10). Presumably the exfoliation of glycogen-laden cells and cell fragments from the peridermal surface will account for a net contribution of glucose to the fluid, since these detached cell portions are rapidly disintegrated in the amniotic fluid (6).

In various mammals the exfoliative capacity of fetal skin in late pregnancy is described (9, 11). It seems as if the extensive exfoliation in the human periderm in early pregnancy is a phenomenon that differs from the detachment of squames from the fetal epidermis

during the last months of pregnancy. The latter process is used as a tool in maturity determination and thus reflects the differentiation of fetal epidermis. An index of fetal maturation, which appears to relate to the crucial lung maturation and is more equivalent to available phospholipid determination methods in predicting fetal maturity. In this phase of human pregnancy the layer responsible for exfoliation to the amniotic fluid is no longer the periderm, rather the germinative portion of the original layered epidermis.

To conclude, there seem to be two distinct exfoliative mechanisms operating in the fetal



The close interrelationship between bundles of tonofilaments and groups of glycogen granules suggests a functional relation to exfoliation capacity of periderm. Transmission electron microscopy magn 90 000  $\times$



Fig 10 Close up of early indentation of human periderm starting an apparent closure of bridging or branching microvilli (arrows). Note uniform shape of microvilli close to which are observed tonofilaments (T) and scattered glycogen granules (G). Transmission electron microscopy magn 90 000  $\times$

human pregnancy. They are not active simultaneously but during two fairly well defined time periods in early and late pregnancy respectively. In early pregnancy a detachment of glycogen stuffed cytotrophic portions prevails while in late pregnancy a detachment of keratinized cells is predominant. The former kind of material can be used for various purposes in early pregnancy while the latter kind can be used for determination of fetal maturity. Both kinds of material are antenatally available tissue material from the fetus whereby the fetal condition may be elucidated.

#### ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (B78-17X), Stiftelsen Allmänna Barnhusets Minnesfond and Prenatalforskningsnämnden.

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*Submitted for publication August 30 1978*

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## ULTRASONIC MEASUREMENT OF FETAL BODY SIZE

## A randomized controlled trial

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**Abstract** A total of 745 Caucasian women were allocated at random between two groups A and B at the first antenatal visit. At the completion of the study 341 patients in group A and 364 patients in group B underwent a single ultrasonic measurement of fetal chest area between 32 and 36 weeks of gestation. The medical staff was not informed of these results. Three hundred and four patients in group B acted as controls. The percentage of clinically detected small for dates (SFD) was low in both groups A (36 per cent) and B (42 per cent). The ultrasonic detection rate of the clinically detected SFD (group A) was 77 per cent. The clinical detection rate of large for dates (LFD) was only 12 per cent in group A and 13 per cent in group B. The ultrasonic detection rate of the clinically unrecognized LFD (group A) was 60 per cent.

was a negative or positive discrepancy in fundal height of two weeks or more or any other abnormal antenatal finding the patient was referred to the Ultrasound Department. In these instances the medical staff was informed of the ultrasonic data obtained.

Ultrasonic measurement of the chest area was performed caudal to the fetal heart pulsations using a sound velocity of 1540 meters per second (10). The 5th and the 95th centile of the chest area curve based on 302 normal pregnancies between 23 and 42 weeks of gestation were used as the lower and the upper limit of the normal range (11). Birth weight was defined as being between the 10th and 90th centiles (normal) below the 10th centile (small for-dates) or above the 90th centile (large for-dates) according to the Tables of Kloosterman (6) correction being made for maternal parity and fetal sex.

In the last couple of years a stream of information has emerged on the value of ultrasonic measurement of fetal head and body size relative to fetal growth. Data indicate that fetal chest and upper abdominal size are more sensitive parameters for the detection of abnormal growth than fetal biparietal diameter (BPD) (1, 2, 3, 4, 5, 8, 10, 11, 12). This paper presents the results of a randomized controlled trial in the significance of a single 3rd trimester ultrasonic measurement of fetal chest area in detecting small and large for-dates infants.

## MATERIAL AND METHODS

A total of 745 Caucasian women was included in the study and were divided into two groups A and B depending on booking number at the first antenatal visit (even numbers to group A, odd numbers to group B). Each patient in group A was sent to the Ultrasound Department for fetal chest area measurement between 32 and 36 weeks of gestation. The medical staff in the outpatient department was not informed of the results. The patients in group B acted as controls.

In both groups antenatal care included a single BPD measurement before 25 weeks gestation in case the onset of the menstrual period was in doubt and assessment of fetal growth by serial abdominal palpation and measurement of the distance between the upper rim of the pubis and uterine fundus using a tape measure. When there

## RESULTS

At the completion of the study group A consisted of 341 and group B of 364 patients since in both groups patients had dropped out due to mid trimester abortion, premature delivery, intra uterine death or omission to refer the patient to the Ultrasound Department. The mean lag between ultrasonic measurement of fetal chest area and date of delivery was 32 days (range 4-71 days) in group A and 36 days (range 2-64 days) in group B. Further results will now be presented in relation to fetal birthweight. Normal birth weight ( $n=603$ ). In group A ( $n=295$ ) 257 patients were scanned solely in relation to the project (non referrals). Fetal chest area was situated below the normal range in 9 and above this in 3 cases.

Thirty eight patients in group A were referred to the Ultrasound Department due to a negative ( $n=26$ ) or positive ( $n=12$ ) discrepancy in fundal height. Fetal chest area fell below the normal range in 3 and above this in 2 cases.

In group B ( $n=308$ ) 18 patients were sent for an ultrasonic examination because of a negative discrepancy, 13 patients because of a positive discrepancy. Fetal chest area fell below the normal range in 2 and above this also in 2 cases.



Table 1 Clinical and ultrasonic detection rates of small for dates in groups A and B

	Group A (n=28)				Group B (n=33)			
	Clinically detected 5-10% <5%		Clinically undetected 5-10% <5%		Clinically detected 5-10% <5%		Clinically undetected 5-10% <5%	
Ultrasonically detected	6	3	10	4	4	9	12	7
Ultrasonically undetected	1		4		1			

never referred to Ultrasonic Department

**Small for dates (n = 61)** Table 1 presents the clinical and ultrasonic detection rates of small for dates (SFDs) for groups A and B

In group A (n=28) the number of clinically detected SFDs was 10 (36 per cent) 9 of these 10 SFDs were also diagnosed by ultrasound

In the 18 clinically missed SFDs birthweight varied between the 5th and 10th centile in 14 and below the 5th centile in 4 cases. In the 5-10th centile weight range 10 SFDs (71 per cent) were recognized during ultrasonic examination. In the weight range below the 5th centile all 4 SFDs were detected by ultrasound

In group B (n=33) the number of clinically recognized SFDs was 14 (42 per cent) 5 (30 per cent) fell in the 5-10th centile weight range (n=17) 9 (56 per cent) were situated below the 5th centile (n=16)

Of these 14 clinically detected SFDs 13 were also diagnosed by ultrasound. Nineteen SFDs remained undetected and were therefore never referred for ultrasonic examination

**Large for dates (n=41)** Table II gives the clinical and ultrasonic detection rates of large for dates (LFDs) in groups A and B

In group A (n=18) only 3 LFDs were detected clinically 2 of these were recognized by ultrasound. In the 15 clinically undetected LFDs weight was situated between the 90th and 95th centile in 9 and above the 95th centile in 6 cases. In the former weight group 5 (55 per cent) LFDs were detected by ultrasound. In group B (n=23) the number of clinically LFDs was only 3 (13 per cent) one situated in the 90-95th centile weight range. The clinically diagnosed LFDs were also detected by ultrasound. Of the 20 LFDs which were missed 11 (55 per cent) fell in the 90th-95th weight range 9 (45 per cent) fell above the weight for gestation

## DISCUSSION

In the normal birthweight range the discrepancy in fundal weight was low in 13 per cent in group A and 9.5 per cent in group B. The percentage of false positive ultrasonic results

Table II Clinical and ultrasonic detection rates of large for dates in groups A and B

	Group A (n=18)				Group B (n=23)			
	Clinically detected 90-95% >95%		Clinically undetected 90-95% >95%		Clinically detected 90-95% >95%		Clinically undetected 90-95% >95%	
Ultrasonically detected	1	1	4	4	1	2	11	9
Ultrasonically undetected		1	4	2				

never referred to Ultrasonic Department

chest area situated outside the normal range) 13 and 10 per cent in the referrals of group A and only 4.5 per cent in the non referrals in group B. The marked difference between the non referred and referred patients may be explained by the fact that in the latter fetal birthweight was often situated far or on the 10th or 90th centile.

The percentage of clinically detected SFDs is disappointingly low in both group A (36 per cent) and group B (12 per cent). This is particularly so in the 5-10th centile weight range. Similar observations have been made by others. MacLaurin (7) stated that only about 10 per cent of those fetuses which ultimately fell below the 10th centile weight for gestation were suspected during the antenatal period. Campbell (2) found that only 41 per cent of a group of 115 babies with a birthweight below the 5th centile were recognized clinically. Tejani (9) stated an even lower percentage of 33 per cent. The ultrasonic detection rate of clinically undiagnosed SFDs (group A) is 77 per cent (5-10th centile weight range 71 per cent, below the 5th centile 100 per cent). The ultrasonic detection rate of the clinically established SFDs is high: 90 per cent in group A and 93 per cent in group B. The clinical detection rate of LFDs was lower: 12 per cent in group A and 13 per cent in group B. This is not surprising since the obstetrician is more aware of a fetus than a positive discrepancy the former is particularly being associated with a raised perinatal mortality and morbidity rate. The ultrasonic detection rate of the clinically unrecognized LFDs was 57 per cent (55 per cent in the 90-95th centile weight range and 67 per cent in the weight range above the 95th centile). This detection rate is somewhat lower than the 80 per cent diagnosed in an earlier study (1).

It can be concluded that the data presented here support the need for routine ultrasonic measurement of fetal size in the early diagnosis of the under- and oversized infant. This is particularly so for the so-called borderline underweights, i.e. in the 5-10th centile weight for gestation.

It is realized that routine ultrasonic scanning of an obstetric population has considerable practical consequences. A single measurement of fetal chest or abdominal size at 32-36 weeks of gestation

would however certainly ease the workload compared with a combined measurement of both fetal body and head size.

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Submitted for publication October 25 1978

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1981

Date	Place	Name	Officer
<b>Continuation</b>			
December 1-4	Kairo Egypt	Second Congress of the International Society for the Study of Hypertension in Pregnancy	Docent Hjördis Robbe Dept of Obstet and Gynecol Karolinska Hospital S-104 01 Stockholm 60 Sweden
December 1-12	Melbourne Australia	Seventh UICC Training Course in Cancer Research	Dr A W Burgess UICC Course The Walter and Eliza Hall Inst. Royal Melbourne Hosp PO Box 123 Victoria Australia
<b>1981</b>			
January 26-31	Mexico City Mexico	Pan American Congress of Andrology	Gerald Bagatzinski Conat Adm 31600 West Chicago Livonia, MI 48150 USA
March 22-26	West Berlin West-Germany	Third World Congress of Human Reproduction	Dozent L. Mettler Frauerkln der Hogewischstr 4 D-2300 Kiel 1
June 9-12	Ostend Belgium	Third International Congress on the Menopause	The International Menopause Soc 8 av Don Bosco 1140 Brussels
August 24-28	Cambridge England	XIII Asia Endocrinologica Congress	Conference Services Ltd XIII Asia Endocrinologica Congress 3 London SW7 4EJ England
Sept-Oct	Athens Greece	Vith European Congress on Sterility (ESCO)	Sekretariat Prof Dr K. Smol klinik der Universität Kiel Hygie- strasse 4 2300 Kiel 1 West Germany
October 23-31	Melbourne Australia	Eight Asian Congress of Obstetrics and Gynecology	The Organizing Secretary Vith Congress of Obstetrics and Gynecology G P O Box 21957 Melbourne Victoria Victoria Australia

## MATERNAL AND FETAL METABOLIC RESPONSES TO RITODRINE IN THE SHEEP

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**Abstract** Ritodrine hydrochloride was infused intravenously for 60 minutes into pregnant ewes or for 29 minutes into lambs during the last third of gestation. The maternal, fetal acid base balance and carbohydrate status was determined prior to, during and after the ritodrine infusions. Ritodrine was also infused into pregnant sheep in labor until further inhibition of uterine activity could be achieved. In these studies, acid base balance was frequently assessed. In maternal or fetal infusion of ritodrine resulted in no changes in maternal or fetal acid base balance during the periods of study. Lactate and pyruvate concentrations in mother and fetus rose during the infusion. The lactate:pyruvate ratio remained stable, suggesting an increase in aerobic and anaerobic glycolysis. Maternal glucose concentrations were significantly elevated after one hour of ritodrine infusions to the ewes, but minimally elevated in the fetus. Fructose concentrations were unchanged. The role as fetal fuels of increased metabolic products of glycolysis caused by beta adrenergic stimulation is discussed.

There is evidence which suggests that beta adrenergic agents may be useful in treating fetal distress (5, 10) possibly by decreasing fetal respiratory acidosis. It has also been reported that fetal growth is improved (20, 3) and that the incidence of respiratory distress syndrome is decreased (2) with the use of maternally administered beta adrenergic agents. However, conflicting reports have also been published concerning the effects of beta adrenergic stimulation on fetal acid base balance. Increased acidosis has been reported in hypoxemic fetuses and this has been attributed to increased glycogenolysis and lysis (9, 21, 13).

View of the uncertain effects of beta adrenergic stimulation on acid base balance and glucose metabolism, we studied the metabolic effects of one of the beta adrenergic agents, ritodrine hydrochloride (Phlips Inc. Lab. Inc. Columbus, Ohio) in the chronically prepared pregnant sheep.

## MATERIAL AND METHODS

**Preparation** We used 43 sheep with 36 singleton and 7 twin gestations. Operation for insertion of maternal and fetal catheters was performed at 100 to 145 days of ges-

tation. The general surgical procedure has been described in detail previously (17, 18). Briefly, polyvinyl catheters were inserted into a maternal and fetal femoral artery and vein and into the amniotic cavity. Following closure of the uterus and abdomen, the catheters, which had been exteriorized to the maternal flank, were filled with heparin and sealed with a metal plug. Kanamycin, 50 mg, was injected into the amniotic cavity for 4 days. The animals were brought daily in mobile cages into the laboratory where the catheters were only flushed or the experiments performed.

**Experimental Design** Experiments were usually performed three to five days after surgical preparation (range 2 to 27 days). The ewes had free access to alfalfa pellets and water during the studies. Prior to any ritodrine infusion, the steady state of the animal was assessed for one to two hours by means of measuring the maternal and fetal heart rate and blood pressure, acid base status, plasma glucose concentration and amniotic fluid pressure. During the experiments, arterial blood pH and gas tensions were measured at 20 to 30 minute intervals. Only one experiment was performed per day.

Ritodrine hydrochloride, diluted in isotonic saline, was infused into either the maternal or the fetal femoral vein using precalibrated infusion pumps. The dose was calculated by estimation of maternal and fetal weight and corrected by the measured weight later. The weight of the ewes ranged from 45.5 to 68.0 kg, and that of the fetuses from 1.1 to 4.0 kg. Paired blood samples for acid base or carbohydrate metabolism measurements were obtained simultaneously from the maternal and fetal femoral arteries.

Studies were performed in the following three groups. **Group A** Ritodrine was infused into 11 ewes in labor. Uterine activity was spontaneous in eight experiments and induced with an oxytocin infusion in the other three. Ritodrine was infused from 40 minutes to 4 1/2 hours at an infusion rate of 1.9 to 4.9  $\mu\text{g/kg/min}$ . The infusion rate of ritodrine was doubled every 20 minutes until no further decrease in uterine activity was observed. Acid base studies were performed before and during the infusion.

**Group B** Ritodrine was infused into 32 ewes not in labor at a mean constant infusion rate of 1.9  $\mu\text{g/kg/min}$  for 60 minutes. Arterial blood samples were obtained prior to, at the end of, and one hour after the ritodrine infusion. Acid base, plasma glucose and blood fructose, lactate and pyruvate analyses were performed.

**Group C** Ritodrine was infused into 19 fetal lambs at an increasing infusion rate until a persistent tachycardia was obtained. The infusion rate was 1.9  $\mu\text{g/kg/min}$  (mean constant infusion rate was 1.9  $\mu\text{g/kg/min}$ ) and the mean infusion rate

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**Table 1** Maternal and fetal arterial blood acid base balances and gas tensions before and during ritodrine infusion into ewes in labor. The ritodrine dose increased every 20 min from 1.9 to 4.9  $\mu\text{g/kg/min}$  until no further inhibition of uterine activity occurred. Mean values  $\pm$  S.E.

	Before ritodrine	During ritodrine at 44 min	During ritodrine at 97 min
<b>Maternal arterial</b>			
pH	7.47 $\pm$ 0.01	7.46 $\pm$ 0.01	7.45 $\pm$ 0.01
PO <sub>2</sub> (torr)	101 $\pm$ 2	96 $\pm$ 2	103 $\pm$ 0.03
PCO <sub>2</sub> (torr)	32 $\pm$ 1	33 $\pm$ 1	32 $\pm$ 1
B.E. (mEq/L)	0 $\pm$ 0.5	0.5 $\pm$ 1.0	-1.5 $\pm$ 1.0
<b>Fetal arterial</b>			
pH	7.14 $\pm$ 0.01	7.33 $\pm$ 0.02	7.31 $\pm$ 0.03
PO <sub>2</sub> (torr)	20 $\pm$ 1	19 $\pm$ 2	19 $\pm$ 1
PCO <sub>2</sub> (torr)	49 $\pm$ 1	50 $\pm$ 2	50 $\pm$ 2
B.E. (mEq/L)	0 $\pm$ 1.0	0 $\pm$ 1.5	-1.5 $\pm$ 1.5
$\Delta$ pH	0.12 $\pm$ 0.01	0.14 $\pm$ 0.02	0.15 $\pm$ 0.03
$\Delta$ B.E. (mEq/L)	0.5 $\pm$ 1.0	1.0 $\pm$ 1.5	0.5 $\pm$ 1.0

Number of animals included in the before ritodrine during ritodrine at 44 min and during ritodrine at 97 min groups were 10 respectively

(range 13 to 50 minutes). Arterial blood samples were obtained and analyzed as described for Group II. Analytical methods: Arterial blood gas tensions and pH were measured at 39°C with Radiometer microelectrodes and base excess (B.E.) then calculated (16). Arterial plasma glucose was determined as the mean of five measurements using a glucose analyzer (Beckman Instruments Inc., Fullerton, Calif.). Whole blood lactate and pyruvate concentrations were measured using the lactic dehydrogenase method (Sigma Chemical Corp., St. Louis, Mo.). Whole blood fructose was determined by the resorcinol method (22). Blood fructose, lactate and pyruvate were assayed from frozen precipitates kept at -20°C in duplicate. The variation coefficients were: plasma glucose 3 per cent, blood lactate 0.9 per cent, pyruvate 7.5 per cent and fructose 15 per cent.

The data were statistically evaluated by the paired *t* test.

## RESULTS

**Group A.** The ritodrine infusion resulted in a 70 per cent decrease in uterine activity. There were no significant changes in maternal or fetal arterial blood pH, gas tensions or B.E. (Table 1). Seven of the fetal lambs had a mean arterial PO<sub>2</sub> of 17 torr during the control period, but the mild hypoxemia did not increase during the ritodrine administration.

**Group B.** When ritodrine was infused into ewes not in labor, no changes were found in arterial blood pH, gas tensions or B.E. during the infusion (Table 1). One hour after completion of the infusion, maternal arterial pH had increased from 7.46  $\pm$  0.01 to 7.48  $\pm$  0.01 (mean  $\pm$  S.E.). Concomitantly the pH gradient from mother to fetus increased significantly ( $p < 0.01$ ) from 0.13  $\pm$  0.01 to 0.16  $\pm$  0.02 in the

recovery period. In these experiments 22 fetal lambs had an arterial PO<sub>2</sub> of 20 torr or greater. All lambs had less than 20 torr. There was no difference between these two groups in their acid responses.

Plasma glucose levels were determined in 22 and 24 fetal lambs. As shown in Fig. 1A, the maternal plasma glucose was slightly elevated after one hour into the ritodrine infusion and was elevated further one hour into the recovery period to a mean of 61.9 mg/100 ml from a control value of 61.9 mg/100 ml. The only significant change in fetal plasma glucose was an elevation from a control of 15.3 to 15.3 mg/100 ml one hour into the recovery phase. Ten of the fetal lambs had an initial plasma glucose below 15 mg/100 ml and the other twelve above that level. The effect in both groups was of a magnitude and the combined results are reported. There were no significant changes in blood glucose concentration in 6 ewes and their fetuses during the ritodrine administration (Fig. 1B).

The maternal lactate concentration increased from 8.8 to 13.5 mg/100 ml (+53 per cent) during the ritodrine infusion and was still elevated (11.5 mg/100 ml) one hour after the infusion (Fig. 1C). Fetal lactate increased from 27.9 to 35.3 mg/100 ml (+27 per cent) during the infusion and remained elevated at 35.3 mg/100 ml one hour later.

The maternal pyruvate concentration also increased during the ritodrine infusion (Fig. 1D). The lactate/pyruvate ratio remained stable (3.4  $\pm$  0.2 before and 3.4  $\pm$  0.2 during and 4.2  $\pm$  0.7 and 4.0  $\pm$  0.5) before, during and after the

Table II Maternal and fetal arterial blood acid base balances and gas tensions before during and one hour after nitrodrine infusion into ewes in absence of uterine activity. Mean dose of nitrodrine was  $1.9 \mu\text{g/kg/min}$  during a mean period of 60 min. Mean values  $\pm$  S.E.

	Before nitrodrine	During nitrodrine	After nitrodrine
Maternal arterial			
pH	$7.46 \pm 0.01$	$7.47 \pm 0.01$	$7.48 \pm 0.01$
$\text{PO}_2$ (torr)	$101 \pm 2$	$101 \pm 2$	$103 \pm 1$
$\text{PCO}_2$ (torr)	$32 \pm 1$	$31 \pm 1$	$31 \pm 1$
pH (mEq/L)	$-0.5 \pm 0.5$	$-0.5 \pm 0.5$	$2.0 \pm 2.0$
Fetal arterial			
pH	$7.33 \pm 0.01$	$7.33 \pm 0.01$	$7.31 \pm 0.02$
$\text{PO}_2$ (torr)	$21 \pm 1$	$20 \pm 1$	$20 \pm 1$
$\text{PCO}_2$ (torr)	$47 \pm 1$	$47 \pm 1$	$48 \pm 1$
pH (mEq/L)	$-1.5 \pm 0.5$	$-1.0 \pm 0.5$	$-2.0 \pm 1.0$
pH	$0.13 \pm 0.01$	$0.14 \pm 0.01$	$0.16 \pm 0.02$
pH (mEq/L)	$1.0 \pm 0.5$	$2.0 \pm 1.5$	$2.0 \pm 1.0$

Number of animals included in the before, during and after nitrodrine groups are 32, 33 and 33 respectively.  
 $105 \quad p < 0.01$

drine infusion (mean  $\pm$  S.E.) Fetal pyruvate increased from  $1.02$  to  $1.23 \text{ mg/100 ml}$  during the infusion and the fetal lactate/pyruvate ratio remained unchanged. This effect was similar in fetal lambs with a maternal  $\text{PO}_2$  above or below  $19 \text{ torr}$  although ewes without hypoxemia had lower initial levels of lactate and pyruvate ( $15.1$  and  $0.75 \text{ mg/100 ml}$  respectively).

Experiment C: Nitrodrine infused directly into the fetus resulted in a small but statistically significant fall in fetal arterial pH ( $-0.01$  units,  $p < 0.05$ ) (Table III). Only other significant change observed was an increase in the base excess difference between mother and fetus. Fourteen normotemic fetal lambs (mean  $\text{PO}_2$   $23 \text{ torr}$ ) responded in a similar manner to five ewes with hypoxemia (mean  $\text{PO}_2$  of  $16 \text{ torr}$ ). No significant changes were noted in maternal arterial pH, base status or blood gas tensions.

Plasma glucose measurements were made in 13 ewes and their fetuses during the nitrodrine infusions and no changes were observed (Fig. 2A). In 6 of these ewes studied in the recovery period there was a slight elevation to a plasma glucose of  $20.6 \text{ mg/100 ml}$  from the control value of  $18 \text{ mg/100 ml}$ . No difference in the responses was found between fetuses from low (mean  $12.7 \text{ mg/100 ml}$ ) or normal (mean  $18 \text{ mg/100 ml}$ ) glucose values.

There were no significant changes in fetal or maternal blood fructose with the fetal infusion of nitrodrine (Table 2B).

With lactate and pyruvate concentrations increased during the fetal nitrodrine infusions (Fig. 2 C & D).

These changes were similar in six normotemic fetuses and in three hypoxemic fetuses. The lactate/pyruvate ratio was  $20.0 \pm 1.6$  during the control period  $19.4 \pm 2.9$  during the infusion and  $20.2 \pm 3.2$  one hour into the recovery period (mean  $\pm$  S.E.). Maternal lactate and pyruvate concentrations remained unchanged.

## DISCUSSION

The acid base balance of the ewe and her fetus underwent no major alterations during the infusion of nitrodrine either into the ewe or the fetus. Although maternal arterial lactate and pyruvate levels were increased, this was not reflected in any major change in maternal arterial pH or  $\text{PCO}_2$ .

While maternal pH is dependent on respiration, metabolic changes and accumulation of organic acids, the fetal acid base balance is also influenced by changes in uterine and umbilical blood flow. We have previously demonstrated that nitrodrine infusions in doses similar to those used in the present studies cause no major change in uterine or umbilical blood flow in sheep (18, 19). The accumulation of organic acids may play a role in decreasing fetal arterial pH. However, although the decrease in fetal arterial pH was statistically significant, the magnitude of the decrease was only  $0.01$  pH units. These findings are in accord with other studies where a  $31 \pm 7$  per cent (mean  $\pm$  S.E.) reduction in uterine blood flow associated with a nitrodrine infusion rate of  $800 \mu\text{g/min}$  for two hours elicited no significant changes in maternal or fetal arterial pH,  $\text{PCO}_2$  or  $\text{PO}_2$  (7).

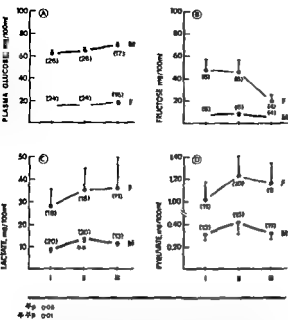


Fig 1 Maternal and fetal arterial plasma glucose (A) blood fructose (B) blood lactate (C) and pyruvate (D) levels before (I) during (II) and one hour after (III) ritodrine infusion into the ewe. Ritodrine dose 1.9 ug/kg/min duration of infusion 60 min. Mean values  $\pm$  SE. Number of animals indicated in parenthesis. M=mother F=fetus

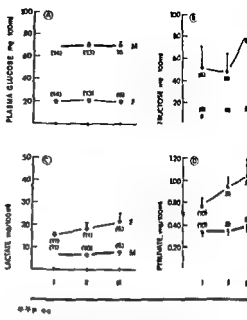


Fig 2 Fetal and maternal arterial plasma glucose (A) fructose (B) blood lactate (C) and pyruvate (D) before (I) during (II) and one hour after (III) nitroprusside infusion into the fetal lamb. Nitroprusside dose 1.9 ug/kg/min duration of infusion 29 min. Mean values  $\pm$  SE. Number of animals indicated in parenthesis. M=mother F=fetus

It has been suggested from studies in humans that the use of ritodrine during labor may improve fetal pH and base excess values (10, 8). In the present investigations in sheep we were unable to demonstrate any improvement in fetal arterial pH or blood gas tensions with inhibition of uterine activity with ritodrine.

Both arterial lactate and pyruvate concentrations in the ewe and the fetal lamb were elevated during the ritodrine infusion and tended to remain elevated for at least one hour after cessation of the infusions. The stable lactate/pyruvate ratios in mother and fetus indicate that the ritodrine infusions stimulated both aerobic and anaerobic glycolysis. Furthermore, the response of the hypoxemic fetal lambs was similar to the normoxemic lambs, even though the initial lactate levels were higher in the hypoxemic group.

It has previously been demonstrated that lactate may serve as an important substrate for fetal metabolism (4, 6) and that lactate may be derived from placental conversion of glucose (4). The present studies, in which there was a relatively small elevation of fetal plasma glucose compared to the more pronounced increases in fetal arterial lactate, could be interpreted as an increase in fetal glycolysis due to the

nitroprusside infusion. It remains to be seen whether the increased fetal lactate served during beta adrenergic stimulation, secondary to placental conversion or due to fetal glycolysis, and to what extent the by the fetus.

The maternal and fetal plasma glucose only moderately elevated during the maternal ritodrine infusion at the doses used. These findings are in agreement with other studies using similar infusion rates (7). The fetal infusion resulted in only a small elevation in plasma glucose in the recovery period. In previous studies, infusion rates two to four times the rates we utilized have been reported to result in significant increases in plasma glucose values from baseline glycemic values (8 mg/100 ml) (7). It is quite possible that with higher infusion rates of ritodrine, over a longer duration, there could be a more significant elevation of fetal plasma glucose levels.

Human studies have indicated that hypoglycemia of the newborn is rarely seen following the use of ritodrine (1). That the beta adrenergic system is involved in the regulation of glucose homeostasis has also been demonstrated by the hypoglycemia reported following the use of beta adrenergic blocking agents (17). L-

le III Maternal and fetal arterial blood acid base balances and gas tensions before during and one hour after ritodrine infusion into fetal lambs. Mean dose of ritodrine was 1.9 µg/kg/min during a mean period of 29 min. Mean values ± S.E.

	Before ritodrine	During ritodrine	After ritodrine
Maternal arterial			
pH	7.48 ± 0.01	7.49 ± 0.01	7.50 ± 0.01
P <sub>O</sub> <sub>2</sub> (torr)	102 ± 3	101 ± 2	101 ± 2
P <sub>O</sub> <sub>2</sub> (torr)	30 ± 1	32 ± 1	31 ± 1
BE (mEq/L)	0 ± 0.5	1.0 ± 1.0	1.5 ± 0.5
Fetal arterial			
pH	7.36 ± 0.01	7.35 ± 0.01	7.34 ± 0.01
P <sub>O</sub> <sub>2</sub> (torr)	21 ± 1	21 ± 1	20 ± 1
P <sub>O</sub> <sub>2</sub> (torr)	44 ± 1	44 ± 1	46 ± 2
BE (mEq/L)	-1.0 ± 0.5	-1.5 ± 1.0	-1.0 ± 1.0
III	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
BE (mEq/L)	0 ± 1.0	2.5 ± 1.0	3.0 ± 1.0

Number of animals included in the before, during and after ritodrine groups are 16, 15 and 13 respectively for mother and 19, 19 and 105 for fetus.

d glucose and lactate in fetal lambs subjected to maternal hypoxemia or epinephrine infusion into the mother have also been reported (15). Thus, it is possible that ritodrine may assist in the response of the fetus to stress in averting hypoglycemia. However, a study is indicated in order to ascertain whether glycogen stores could be depleted and thus potentially adversely affect the response of the fetus. Glucocorticoid administration in rabbits has been shown to increase glucose incorporation into phosphatidyl choline by promoting glycogenolysis (11). The observation, plus the release of free fatty acids by beta adrenergic stimulation (21, 13) may be a factor between the reported decreased incidence of respiratory distress syndrome and the use of beta adrenergic drugs.

In summary, the ritodrine infusions resulted in maternal and fetal metabolic changes similar to those seen during stress but without resulting in any adverse acid base balance changes. It remains to be determined to what extent the fetus is able to utilize increased metabolic products of carbohydrate metabolism.

#### ACKNOWLEDGEMENTS

We wish to thank Miss Françoise Mauray and Mr. Carl Walters for their skillful technical assistance and Dr. J. Hoffman for his guidance with statistical analysis.

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*Submitted for publication June 6 1978*

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## APPENDIX

Dr Anja S I Sumes is recipient of a Fellowship from the Bay Area Heart Association

The research has been supported by USPHS Grant 5R01-06619

The paper has been presented in part at the 2nd Meeting of the Society for Gynecologic Investigation

## SHORT COMMUNICATION

THE USE OF PULSED HIGH FREQUENCY THERAPY (CURAPULS)  
IN GYNECOLOGY AND OBSTETRICS

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curapuls device can be used to give both ultra  
t wave diathermy and athermic pulsed high fre  
quency therapy The biological effects of the latter  
be regarded as the same as those of ultrashort  
diathermy with the difference that no continu  
-elevation of temperature ensues (2) We investi  
i earlier the effect of pulsed high frequency  
therapy on vulval epithelial lesions and noted a bene  
ficial effect in most cases (1) We have now studied  
the use of curapuls in other gynecological and also  
obstetric indications

## RESULTS AND DISCUSSION

The diagnoses and the changes occurring in the pa  
tients symptoms during pulsed high frequency  
therapy are presented in Table I The response to  
therapy appears to have been good in parametrop  
athy eight of 10 patients were either symptomless  
or had improved following therapy The symptoms of  
most of the patients in the other groups with the ex  
ception of endometriosis had also either disappeared  
or diminished during treatment

No significant differences were demonstrated in in  
volution of the uterus after Caesarean section be  
tween the patients given pulsed high frequency  
therapy and the control group

Pulsed high frequency therapy appears to have a  
beneficial effect in various painful conditions located  
to the pelvic region Good results were achieved in  
the treatment of spastic parametropathy a condition  
characterized by troublesome lower abdominal and  
lumbosacral pain where the parametria are tense and  
tender This painful condition is often of long dura  
tion and resistant to spasmolytics and other medica  
tion Curapuls therapy appeared to exert a favorable  
action also in postoperative conditions The possible  
effect of high frequency therapy on the scar forma  
tion of the Caesarean section wound must be left to  
future studies

## SUBJECTS AND THE MODE OF TREATMENT

Pulsed high frequency therapy was administered to 71 gynecological pa  
tients whose mean age was 44.2 years (range 19-72)  
The patients were classified by diagnosis Table I  
Pulsed high frequency therapy in cycles of 10-15 single  
pulses at intervals of two days was administered using  
curapuls device A pulse repetition frequency of 62 Hz  
300 W was used The patients were examined before  
curapuls therapy and two weeks after treatment  
Furthermore we studied the effect of pulsed high fre  
quency therapy on involution of the uterus after cesarean  
section in five women the size of the uterus being  
measured by means of ultrasound and compared these  
five control patients who received no therapy

Table 1 Patients treated by pulsed high frequency therapy

Disease	Total	Symptomless	Better	Unchanged
Spastic parametropathy	10	6	2	2
Postoperative parametropathy	9	3	5	1
Endometriosis	5	—	2	3
Postoperative status	16	9	4	3
Radiotherapeutic status	8	2	4	2
Lower abdominal pain (cause unknown)	11	10	9	4
Total	71	30	26	15

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*Submitted for publication June 6 1978*

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## SHORT COMMUNICATION

## THE EFFECT OF CERVICAL ENCERCLAGE ON UTERINE DIMENSIONS

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Observation of women who underwent cerclage of the uterine cervix in pregnancy indicated that during the 24 hours after operation a marked increase of the uterine size takes place. This feature came rather as a surprise, seventy-one women were studied carefully.

## MATERIAL AND METHODS

Twenty-one patients of gestational ages 12-17 weeks who had a history of preterm delivery or incompetence of the internal cervical os at the Government Hospital Jaffa during the period November 1976 through August 1979 were investigated.

Cerclage was carried out according to the method described by McDonald (1957). A single tobacco-pouch suture was placed approximately one cm below the internal cervical os. Under general anesthesia a purse string suture of silk n. 3 carried on a Mayo needle was in the cervix taking four bites of cervical tissue. Prior to the operation the urinary bladder was emptied and the patient in lithotomy position. The upper part of the symphysis pubis and the border of the uterine fundus were thoroughly palpated and marked with a dye on

the abdominal skin. The midline distance between the two lines was measured in cm and recorded.

The same procedure was repeated 24 hours after operation. All the measurements were carried out by the same doctor. In twenty-five cases the height of the uterine fundus was measured and recorded independently by two doctors and the results compared. Differences in the measurements recorded by the two independent doctors were not significant.

All patients were kept in bed for 24 hours after the operation until the second measurement had been carried out. To exclude the possibility of the results obtained in the study group being due to the bed rest alone, measurements of uterine height were carried out in a control group of women of similar gestational ages who did not undergo cerclage. In the control group a 24-hour period of bed rest did not cause any change in the height of the uterine fundus.

In ten cases an attempt was made to measure the sizes of the uterine cavity and the thickness of the uterine wall using ultrasonography, but we were unable to obtain accurate measurements because of the difficulty of identifying the same uterine plane in the repeat examination. The results suggested, however, that the size of the uterine cavity itself remained unchanged after the operation.

Twenty-one women were checked independently a fortnight later and the expected and actual uterine sizes corresponded.

## 1 Mean differences in uterine size before and after cerclage

No. of patients	Parity	Gestational age (in weeks)	Mean distance between symphysis pubis and uterine fundus (cm)		
			before cerclage	24 hours after cerclage	mean difference (cm)
	0-3	12	8.84	11	2.16
	0-3	13	9.79	11.97	2.17
	0-4	14	8.78	10.92	2.14
	2-3	15	9.2	10.79	1.59
	2	16	10	12	2
	1	17	10.25	12	1.75

# RESULTS

The size of the uterus following cerclage as measured by the distance between the symphysis pubis and the upper board of the uterus was increased in all 71 patients (Table 1). The mean increase was 1.97 cm, S.D.  $\pm 0.22$  cm. The median increase was 2 cm. The postoperative increase in the height of the uterine fundus was not dependent on the pre-operative size. No correlation was found between the degree of the post cerclage 'uterine enlargement' and the gestational age or parity of the mother.

# COMMENTS

Recent work (Bibby *et al.* 1979) has demonstrated that during the early part of the second trimester of pregnancy cervical encirculation is associated with a rapid significant rise of 13-14 dihydro-15 keto prostaglandin F (PGFM) which reflects an increase in intra uterine production of prostaglandin F<sub>2α</sub> which in turn is known to influence capillary permeability (Vane 1971).

Since ultrasonographic investigation indicates change in the size of the uterine cavity we think that the increase in height of the uterine fundus following cerclage is due to a thickening of the wall resulting from congestion.

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Submitted for publication November 15 1979

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## LETTER TO THE EDITOR

## STATISTICAL COMPARISON IN A CONTROLLED TRIAL OF ELECTIVE INDUCTION OF LABOR

Dear Sir

Tylleskär *et al* (2) recently reported some findings from a prospective randomized study on induced versus spontaneous labor dealing with the effects on the mother and the fetus. They concluded that given head entation, normal pregnancy and careful selection of patients with respect to the length of pregnancy, the condition of the cervix, induction of labor using the Cardiff Infusion System with intrauterine pressure recordings and continuous fetal heart rate monitoring is not associated with any increased risks to the mother or fetus during labor and delivery. The authors based their conclusions on statistical analysis applied to a variety of outcomes where no significant differences were found. They reported mean values, standard deviations and p-values for their measurements and tests on the varied outcomes. In their words they reported on the probabilities of discovering differences between the two groups when in fact there are none. However, nowhere in the report do the authors discuss the possibility of overlooking a real difference between the two groups. We think this is a crucial omission, particularly in studies like the one under discussion where the authors conclude that there are no differences between the two groups. Unfortunately, this type of omission is too often missing in similar reports (1).

In order to illustrate the importance of these considerations, we shall take a closer look at a few of the findings reported by Tylleskär *et al*. The analysis is based on comparison of variable outcomes for 84 women: 43 with induced labor and 41 delivered spontaneously. We shall focus on two comparisons reported in Tables IV and VI in their paper and will restrict our discussion to the findings reported for primiparae.

Comparison 1 concerned uterine activity at 6 cm cervical dilatation expressed in Montevideo units, which for the induction group of 20 cases was found to be  $158 \pm 58$  and for 15 of the spontaneous group  $152 \pm 51$ .

Comparison 2 concerned the amount of bleeding in ml during the third stage of labor, which for the two groups was  $376 \pm 216$  and  $332 \pm 242$  respectively.

For both these comparisons the authors concluded that there were no statistically significant differences between the induced and the spontaneous groups.

We have computed the chances of concluding that there is no difference between the two groups when in fact there is a real difference, and have related these chances to the magnitudes of such true differences. These relationships are shown in Table 1.

From the table it is seen that in the reported study with a chosen significance level of 0.01, there is a 50 per cent chance of wrongly concluding that there is no difference between the groups, even if there were a real difference in uterine activity of 47 Montevideo units (31 per cent of the observed mean value in the spontaneous group) and a real difference in bleeding of 183 ml (54 per cent of the mean value in the spontaneous group). In order to have only a 10 per cent chance of making similar mistake, the true differences have to be at least 72 Montevideo units and 282 ml respectively. The corresponding differ-

Table 1. Probability of wrongly postulating 'no difference' for the sample sizes and results presented by Tylleskär *et al* (2).

	Probability			
	0.10	0.25	0.50	0.75
<b>Comparison 1</b>				
Uterine activity (M U)				
Sign. level $p < 0.01$	72	61	47	32
Sign. level $p < 0.05$	58	45	32	20
<b>Comparison 2</b>				
Bleeding (ml)				
Sign. level $p < 0.01$	282	236	183	126
Sign. level $p < 0.05$	225	175	126	76

ences are somewhat reduced if one decides on a significance level of 0.05.

This discussion can of course be extended to all the other results in which no significant differences were found.

This should make it abundantly clear how cautious one should be when discussing the findings and drawing conclusions based on relatively small scale studies even though they may be carefully designed and conducted.

We think it is urgent to stress this fact and to encourage authors to include in the discussion of their findings considerations of the type mentioned here.

May 14 1980

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*Submitted for publication May 14 1980*

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## DURATION OF THE SECOND STAGE OF LABOR

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**Abstract** The second stage of labor defined as the time from full dilatation of the external os to delivery of the fetus was recorded during a three month period in 635 labors with vaginal delivery. The median duration in labors of spontaneous onset was 31.3 minutes in para 0 mothers, 14.3 minutes in para 1 mothers and 11.7 minutes in para 2+ mothers. In induced labors the second stage had approximately the same length as in labors with spontaneous onset. Time distribution showed that the second stage in para 0 mothers had a plateau in the 17.5-37.5 minute range whereas para 1+ mothers had a sharper peak at 7.5 minutes. Forty and 45 minutes respectively seemed to be limits at which only very few second stages of labor lasted. Relative delivery terminated 18 per cent of para 0 labors, 6 per cent of the para 1+ labors entering the second stage. The operative interventions seemed to appear in two groups for each parity group. It appeared that fetal asphyxia requiring intervention was discovered before 40 minutes in the second stage in para 0 and before 30 minutes in para 1+ mothers. Later operative termination was more often performed to relieve fatigued mothers.

In a previous study from Akershus Central Hospital the median duration of labor with spontaneous onset was found to be 11 hours 14 minutes for para 0, 4 hours 30 minutes for para 1 and 4 hours 49 minutes for para 2+ women. The second stage defined as the time from full dilatation of the external os to delivery of the child accounted for 16, 10 and 8 minutes of the total durations respectively (2). The data were analyzed by using electronic processing of information continuously collected through specially designed partograms (1). On the partograms the key measurements of the process of labor were marked to the nearest minute of an hour except for the time of delivery which was exact. While the results are undoubtedly valid where the total duration of labor and its first stage are concerned, the rounding error may have influenced the duration of the second stage. Three current Scandinavian textbooks of obstetrics give widely different figures for the average duration of the second stage of labor in nullipara varying from 0.5 to 3 hours

(3, 4, 5). To obtain a better estimate of the true duration of the second stage of labor we designed the following study.

## MATERIAL AND METHODS

The study took place during three months from January to April 1978. For every birth the length of the second stage of labor was specially recorded from the time of a fully dilated cervix to the delivery of the child or children. Other relevant information such as spontaneous or induced labor, epidural analgesia, fetal presentation, operative delivery, weight of the newborn and Apgar score was also noted on special forms which were filled in by the midwives.

The two participating hospitals were Akershus Central Hospital (ACH) and Gjøvik Hospital (GH). The obstetrical department of ACH has three senior and 9 junior doctors while the staff at GH has two gynecologists for which reason collaboration with the surgical department is necessary. Uncomplicated labors and deliveries are handled by midwives in both hospitals.

The total number of births during the study was 183 at GH and 531 at ACH (Table I). Of these 60 were cesarean sections with no second stage of labor and 7 twin births which were excluded from this study. Table I shows that the proportion of cesarean sections was higher at GH than at ACH (11.5 against 7.3 per cent) and that the difference was due to more frequent use of cesarean sections during labor at GH, the percentages of elective operations being equal.

In twelve cases the length of the second stage was not recorded. This was due to heavy work load and we do not think that the omission of these cases has biased the results. The analysis comprises 635 labors of which 243 (38.3 per cent) were in primipara (para 0) and 392 (61.7 per cent) in mothers who had given birth before (para 1+). Labor was induced by oxytocin in 91 (14.3 per cent), the rest being labors with spontaneous onset.

The type and frequency of operative intervention during the second stage of labor are shown in Table II. Labor terminated spontaneously in 82.3 per cent of para 0 and in 94.4 per cent of para 1+ mothers. In the study group there were no cases of fetal death before the onset of labor.

In presenting the findings of the study the results are being pooled. However, separate analysis of data from the two hospitals revealed no differences in any of the parameters studied.



Table I Births during study period 16th January–15th April 1978

	Gjøvik Hospital		Akershus Central Hospital		Both institutions	
	No	%	No	%	No	%
Second stage recorded	157	85.5	478	90.0	635	88.9
Second stage completed but not recorded	2	1.1	10	1.9	12	1.7
Cesarean section during first stage	13	7.1	15	2.8	28	3.9
Cesarean section elective	8	4.4	24	4.5	32	4.5
Twin births not recorded	3	1.6	4	0.8	7	1.0
Total	183	100.0	531	100.0	714	100.0

## RESULTS

The average duration of the second stage of labor is shown in Table III. It is seen that the second stage lasts about 30 minutes in para 0 mothers both for labors of spontaneous and non spontaneous onset. In mothers who had given birth previously (para 1+) the duration was about 15 minutes or half that in mothers who had their first delivery. In labors with spontaneous onset para 2+ mothers had slightly shorter second stage than para 1 mothers 11.7 minutes and 14.3 minutes median duration respectively. In induced labors the number of para 2+ mothers was very small and did not allow for separate analysis.

By studying the distribution of the length of the second stage of labor in spontaneous and induced labors no noticeable difference was found neither in para 0 nor in para 1+ mothers. The cumulative and frequency distributions are therefore shown together for spontaneous and induced labors in Fig. 1. As shown in the figure the bulk of para 0 mothers have a second stage length of between 15 and 40 minutes

while para 1+ mothers have a sharper peak in interval 5 to 10 minutes. Very few second stage longer than 45 minutes particularly in para 0 mothers. The longest duration of the second stage recorded in this study was 107 minutes for one mother and 70 minutes for a para 1+ mother. This visualizes the point made by Table III that the duration of the second stage is slightly longer than median duration. This is due to the skewed distributions with long tails to the right.

The distribution of operative interventions in the second stage by time interval showed two peaks for both para 0 and for para 1+ mothers seen in Table IV. The first peak of intervention between 10 and 40 minutes in para 0 and before 30 minutes in para 1+ mothers is thought to reflect distress whereas the second peak at 40+ minutes reflects exhausted mothers with prolonged stages. This assumption is being supported by the distribution of low Apgar scores which had a peak responding to the first intervention peak both in para 0 and para 1+ mothers.

Table II Incidence of intervention in the case study by parity

Type of intervention	Para 0		Para 1+	
	No	%	No	%
None	200	82.3	370	94.4
Vacuum extraction	26	10.7	17	4.3
Forceps	13	5.4	2	0.5
Cesarean section	3	1.2	0	0
Assisted breech	1	0.4	3	0.8
Total	243	100.0	392	100.0

Table III Average duration of the second stage of labor (minutes) by onset of labor and by parity

Onset of labor	Parity	Average duration	
		No	Median
Spontaneous	Para 0	213	31.3
	Para 1	745	14.3
	Para 2+	86	11.7
Induced	Para 0	30	17.5
	Para 1+	61	14.4

average duration in minutes

## IV Distribution of interventions during the second stage of labor by time interval and by parity

of cases	Time interval from beginning of second stage (minutes in per cent)						
	0-9	10-19	20-29	30-39	40-49	50-59	60+
Para 0	—	16.3	11.6	18.6	—	14.0	39.5
Para 1+	13.6	18.2	22.7	9.1	9.1	13.6	100.0

was considered to be of interest to see if the duration of the second stage of labor can be predicted by the total duration of labor. For para 0 mothers there was a slight but definite correlation between these parameters, the coefficient of correlation being 0.4. For para 1+ mothers the coefficient of correlation was close to zero ( $r=0.0529$ ). The length of the second stage of labor was also related in relation to the birth weight of the child. In children weighing less than 3 000 grams there was a slight tendency towards a shorter second stage.

## DISCUSSION

The length of the second stage of labor is in many instances determined by operative intervention. This was the case with 17.7 per cent of para 0 mothers and 13.6 per cent of para 1+ mothers in this study. Some elective cesarean sections were performed in cases where a prolonged second stage may be anticipated. Due to such interferences a true biological picture of the second stage cannot be obtained. The error introduced by operative deliveries is mainly a shortening

of the mean values in Table III. The median values which give the time when 50 per cent have completed the second stage will however be less affected.

We found the second stage of labor to last somewhat longer than in the previous computer based study on the duration of each stage of labor (2). The probable reason for this discrepancy is the rounding error previously mentioned. The average lengths found about 30 minutes for para 0 and 15 minutes for para 1+ mothers are still low in relation to statements in two of the quoted textbooks (3, 4).

The time-distribution curves for para 0 and para 1+ mothers (Fig. 1) were different in shape. Para 0 mothers had a long flat plateau in the 17.5-37.5 minute range while para 1+ mothers showed a peak at 7.5 minutes followed by a smooth fall. Forty and 45 minutes seemed to represent limits beyond which only very few second stages lasted.

Operative interventions in the second stage of labor seemed to appear in two different clusters for each parity group. The data sets were small, however both groups showed the same pattern. When this was coupled with the distribution of low Apgar scores (not tabulated) it appeared that fetal asphyxia requiring intervention was discovered before 40 minutes in para 0 and before 30 minutes in para 1+ labors. Operative termination beyond these limits was more often performed to relieve fatigued mothers.

The second stage of labor is the period of greatest physical strain on both mother and fetus. Operative delivery upon signs of life threatening fetal asphyxia is mandatory. In Norway it is our impression that the attitude in many maternity institutions in prolonged second stages with no signs of fetal asphyxia is to wait and see. This may have profound ill-effects on the mothers who in many cases look back upon their labors as a shocking experience which they never want to repeat. The question of when to intervene in prolonged labor can be resolved on the basis of the time-distribution graphs of the second stage. It appears that 45 and 35 minutes are reasonable limits in para 0 and para 1+ labors respectively.

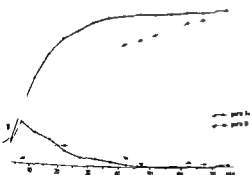


Fig. 1. Frequency distributions and cumulative distributions of the duration of the second stage of labor in 243 para 0 and 39 para 1+.

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*Submitted for publication November 14 1979*

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## METABOLIC EFFECTS OF INTRAVENOUS RITODRINE INFUSION IN PREGNANCY

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The effects of intravenous administration of Ritodrine on blood glucose insulin electrolytes plasma pH and red cell potassium and venous pH were investigated. A highly significant negative correlation between blood glucose and red cell potassium concentration was registered (96.68).

Significant changes during Ritodrine infusion were observed in serum levels of sodium chloride calcium phosphate and magnesium. pH did not seem to be influenced by intravenous Ritodrine administration. Cardiac arrhythmias or electrocardiographic deterioration occurred.

Intravenous Ritodrine administration seems to be safe in normal pregnancies. A risk may be present in diabetic pregnant patients and pregnant women being treated with diuretics.

Ritodrine, a potent beta adrenergic agent is being increasingly used in obstetrics to suppress uterine contractions in the management of premature labor (2, 8).

Although the metabolic effects of this drug have not been thoroughly evaluated, constant findings of an increase in blood glucose and insulin levels and a decrease in serum potassium during intravenous Ritodrine administration have been reported (3, 6, 7, 13). The purpose of this investigation was to determine the clinical and metabolic effects of intravenous infusion of Ritodrine. Specially, correlations between maternal carbohydrate metabolism, ionic equilibrium of potassium and electrocardiographic findings were investigated.

## MATERIAL AND METHODS

The study group consisted of ten patients considered at risk for premature labor because of previous preterm delivery with or without cervical incompetence, placenta previa, or premature uterine contractions. Patients with a familiar history of diabetes mellitus were not included in the

The characteristics of the patient population are shown in Table I.

Normal saline infusion was begun via a scalp vein set #18 at a rate of 1 ml/min. Blood samples were collected from the collateral antecubital vein both at the beginning of the infusion and after 30 and 60 minutes. After one hour Ritodrine was administered by an infusion pump at a constant rate of 200 µg/min. Blood samples were collected at 30, 60, 120 and 180 minutes during the Ritodrine infusion. The samples were divided with one part immediately serving to measure glucose (Technicon Auto-Anal N16b) electrolytes (Eppendorf flame photometry) and potassium levels in erythrocytes. Red cell potassium was calculated according to the following formula:

$$K_{RBC} = \frac{K_H \cdot K_P (1 - Htc)}{Htc}$$

where  $K_{RBC}$  = potassium concentration in red blood cells  
 $K_H$  = potassium concentration in hemolysate  
 $K_P$  = potassium concentration in plasma  
 $Htc$  = hematocrit

Hemolysis was achieved by adding two parts of distilled water to one part of whole blood.

The other part of each sample was stored in deep freezer at 20 °C for insulin assay by RIA method (Sorin France).

Heparinized blood samples were immediately analyzed for pH values (Radiometer Copenhagen BMS3 Mk.).

Urine collected before and after the second and fourth hour of infusion was analyzed for potassium (Atomic absorption spectrophotometer Techtron M100) and creatinine (Jaffy reaction).

Pulse rate and blood pressure were measured after each blood sample collection and electrocardiograms were performed in all patients at time 0 and during the third hour of Ritodrine infusion.

In all patients blood glucose, insulin, serum and erythrocyte potassium levels were again determined 24 hours after the infusion.

Table I Characteristics of patient population

Number of patients	10
Age (in years)	23.8 ± 4.8
Nulliparae	4
Primiparae	6
Singleton pregnancy	8
Twin pregnancy	2
Weeks of gestation	30.6 ± 2.8

Table II Mean values and standard deviations (mmol/l and pH units respectively) of the parameters gated during saline and ritodrine administration Time in minutes before and after 0 time

	-60	-30	0	+30	+60	+120	+180
Glucose	4.12±0.41	4.13±0.2	4.07±0.31	4.70±0.46	5.22±0.56	5.61±0.36	5.87±0.41
Insulin	88.2±37.6	93.2±28.4	96.8±79.4	205.9±59.5	253.3±7.5	230.3±63.5	229.5±31
K <sub>s</sub>	3.77±0.39	3.65±0.3	3.65±0.37	3.17±0.42	2.85±0.37	2.73±0.34	2.64±0.3
K <sub>RBC</sub>	91.7±1.67	91.9±2.40	91.9±0.77	93.2±2.95	93.9±1.96	93.8±2.14	93.1±1.1
Na	141.0±2.65	140.0±3.16	140.2±3.48	140.4±3.97	141.7±2.45	141.2±4.54	142.3±1.1
Cl	101.7±3.6	99.8±3.1	100.8±3.8	99.8±3.4	101.2±3.8	101.8±3.6	101.4±3.3
Ca	2.24±0.12	2.20±0.10	2.24±0.11	2.20±0.15	2.19±0.12	2.16±0.12	2.15±0.1
Pb	1.07±0.31	0.97±0.21	0.97±0.18	0.91±0.20	0.96±0.27	0.93±0.16	0.92±0.1
Als	0.85±0.19	0.86±0.19	0.85±0.15	0.84±0.15	0.76±0.24	0.75±0.16	0.74±0.1
pH	7.25±0.06	7.29±0.03	7.32±0.03	7.30±0.05	7.30±0.06	7.29±0.10	7.31±0.1

## RESULTS

The mean values and standard deviations of all the parameters which were investigated are presented in Table II

Blood glucose values were not influenced by saline infusion, however they rose significantly during Ritodrine infusion from a mean value of 4.12 mmol/l to 5.91 mmol/l (Fig. 1) ( $t < 0.005$  in all instances). Blood glucose levels taken 20 hours thereafter returned to preinfusion values (4.16 mg/dl).

Blood insulin levels were significantly increased 30 and 60 minutes after Ritodrine infusion ( $t < 0.005$ ) thereafter an insignificant decrease in insulinemia occurred (Fig. 2).

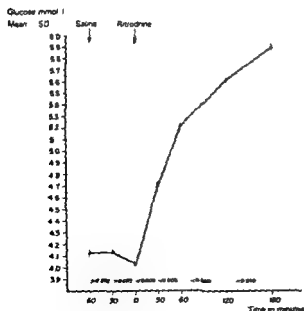


Fig. 1 Glycemia mean  $\pm$  SD during saline and ritodrine infusion

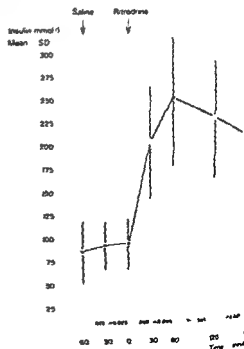


Fig. 2 Insulinemia mean  $\pm$  SD during saline and ritodrine administration

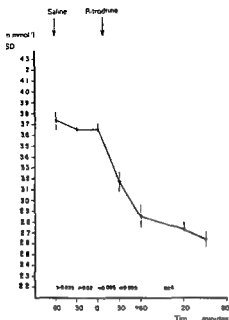


Fig 3 Potassium levels mean  $\pm$  SD during saline and ritodrine infusion

infusion red cell potassium showed a highly significant rise ( $t < 0.005$ ) thereafter an insignificant rise being observed (Fig 4). The correlation coefficient between plasma and red cell potassium was highly significant ( $r = -0.962$ ) (Fig 5).

Similarly a highly significant negative correlation was found between serum glucose and potassium level while a positive correlation ( $r = +0.983$ ) was recorded between insulinemia and red cell potassium concentration.

The other ions investigated were sodium, chloride, calcium, phosphorus and magnesium. No influence on these parameters was noted by saline or by Ritodrine infusion. The venous pH was not influenced by saline or Ritodrine infusion.

No significant difference in the urinary potassium excretion was found.

Clinically an increase in the heart rate was observed in all patients. No arrhythmias or alterations in the ECG were observed.

## DISCUSSION

Adrenergic agonists activate adenylate cyclase, an enzyme which catalyzes the conversion of ATP to cyclic AMP, resulting in an accumulation of intracellular cyclic AMP (10). Cyclic AMP both activates hepatic

glycogen phosphatase and inactivates glycogen synthetase, resulting in an increased output of glucose from the liver (5).

Blood insulin elevation occurs probably as a result of the cyclic AMP mediated stimulating effect on the pancreatic beta cells (1, 9) and as a result of an indirect influence due to the elevated blood glucose levels.

While the observed elevation in blood glucose seems not to be a risk in normal patients with an adequate pancreatic reserve, such changes assume importance in the diabetic patients. Three cases of diabetic pregnant women treated with Ritodrine showed a significant increase in insulin requirement during the infusion (4, 14).

Previous investigation has found that serum potassium drops abruptly during Ritodrine infusion (7). This hypokalemic action of Ritodrine may be secondary to an elevated blood glucose, and the increased insulin secretion; however, we have been unable to find any data concerning the metabolic fate of the potassium lost actively from the plasma space. The present study correlates the elevated red cell potassium levels with lowered serum levels, suggesting that potassium lost to the plasma space actively enters the erythrocyte compartment.

In the average pregnant woman in the third trimester the total plasma volume would amount to 4 000 ml, and the red cell volume would be approximately

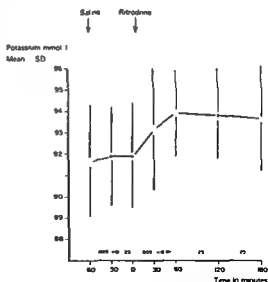


Fig 4 Potassium concentration in red blood cells mean  $\pm$  SD during saline and ritodrine infusion

■ Red Blood Cells  
mmol/l

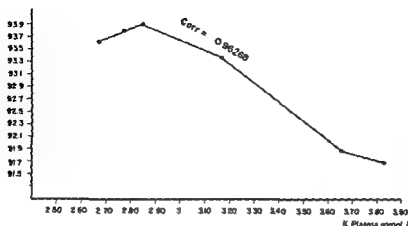


Fig 3 Correlation<sup>2</sup> between plasma and red potassium levels

1 800 ml (12). With the mean maximal drop in serum potassium taken as 1 mmol/l the average total loss of potassium from the plasma space would be 4 mmol. On the basis taking the average maximal increase in red cell potassium (1.9 mmol/l) the total gain of potassium to the red cell space would amount to a very similar quantity of 3.42 mmol. This would suggest that the potassium loss from the plasma space under Ritodrine infusion could be accounted for by migration into the red cells.

As indicated above our results did not show any clear cut alteration in urinary potassium during Ritodrine infusion a finding which precludes enhanced urinary excretion as a cause for the observed decrease in serum potassium levels.

To our knowledge this is the first report indicating a dynamic and reciprocal increase in red cell potassium in the presence of a decreasing serum potassium under the effect of Ritodrine infusion. Both serum and red cell potassium concentrations were found to be at preinfusion levels 20 hours following its termination. Information is lacking concerning the dynamic behavior of both these parameters during the period following the infusion.

The acute and significant hypokalemia manifested during Ritodrine infusion may be of great clinical importance in some selected cases such as in digitalized patients where it may result in deleterious effects. Another clinical situation which deserves special attention under such conditions is represented by patients treated with diuretics. In these cases Ritodrine infusion may be superimposed on an established hypokalemic state increasing the hazard of a severe acute hypokalemia.

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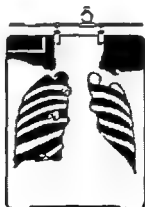
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# **DEPO-PROVERA PROVERA TABLETS**

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# A COMPARISON OF THE ANALGESIC EFFECTS OF METHOXYFLURANE NITROUS OXIDE AND NITROUS OXIDE ALONE DURING LABOUR RELATED TO THE EYSENCK PERSONALITY INVENTORY TEST

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**Abstract** One hundred and thirty three parturients who had received either methoxyflurane nitrous oxide or nitrous oxide analgesia with or without pudendal block underwent Eysenck Personality Inventory Test on the second postpartum day and evaluated their memory of the pain (Subjectively Evaluated Pain Suffering Scores) during labor. Parturients who had received methoxyflurane nitrous oxide analgesia reported significantly lower pain suffering than parturients who had had nitrous oxide analgesia. Subdivision according to Personality Inventory factors showed that at the introvert end of the Extroversion-Introversion scale, methoxyflurane nitrous oxide analgesia without additional pudendal block resulted in significantly lower pain suffering than did nitrous oxide analgesia. On the other hand, nitrous oxide analgesia without additional pudendal block gave significantly lower pain suffering at the extrovert end of the scale.

Among the extroverts there was a tendency, though not statistically significant, towards non approval of the pudendal block.

There are many favourable reports on the analgesic effects of methoxyflurane alone or in combination with nitrous oxide (2, 5, 6, 11, 13, 14). However, none of these reports considered differences in personality when evaluating the differences in analgesic effect.

The purpose of the present study was to look for differences in analgesic effect between the combination of methoxyflurane and nitrous oxide and nitrous oxide alone during labour and to relate the findings to the results of the Eysenck Personality Inventory test (1) (7).

## MATERIAL AND METHODS

One hundred and thirty three parturients were studied. Methoxyflurane nitrous oxide-oxygen analgesia or nitrous oxide-oxygen analgesia was given during alternating three-week periods. Pudendal block was added by the midwives when found necessary.

The parturients received either

- Nitrous oxide 70 per cent in oxygen from a Dedolator (AGA) ( $n=28$ )
- Method a) supplemented with pudendal block anaesthesia ( $n=21$ )
- Methoxyflurane 0.1-0.3 per cent from a Cyprane vapourizer in 50 per cent nitrous oxide in oxygen from a Juno Mark II analgesia apparatus (Mediada) (6) ( $n=37$ )
- Method c) supplemented with pudendal block anaesthesia ( $n=47$ )

All parturients in the study received 50 mg Atarax<sup>®</sup> (Hydroxyzine) and 50 mg Pethidine intramuscularly before the start of inhalation analgesia in accordance with the routine at our clinic. No other drugs were used. Inhalation analgesia was started when the cervix had dilated 4-5 cm in primigravidae and 3-4 cm in parous women. Pudendal block was performed by the midwives using a Kobac needle and 1 per cent mepivacaine solution (Carbocaine<sup>®</sup>). Pudendal block was deemed successful when the parturient experienced anaesthesia in the perineal region. There were no significant differences between the four analgesia groups with respect to age and parity.

On the second postpartum day the parturients were submitted to the Eysenck Personality Inventory Test (7) analyzed by a psychologist (I Å) from the Psychiatric clinic of the University Hospital. On the same occasion the parturient assessed her memory of the pain suffered during labour by putting a mark on a vertical line between two points: no pain (0 scores) and unbearable pain (100 scores). This test was conducted by a test leader who only handed the papers to the parturient and instructed her. The test leader did not know the type of analgesia used during labour and did not evaluate the results.

A rather crude estimation of the course of labour was done retrospectively by one of the authors (B E D) according to criteria shown in Table 1. The course was graded so that a short uncomplicated normal delivery received a score of 0 and a long labour complicated by vacuum extraction and episiotomy or anal sphincter rupture received a high score (maximum 17).

All statistical calculations were performed according to least square methods (Student's *t* test, analysis of variance, analysis of regression). Weighted means were calculated using standardized weights.

Table I Medical assessment of the difficulty of the labor

Stage		Score
Stage 1	Cervical dilation 2-5 cm	
	0-2 hours	0
	2-4 hours	1
	4-6 hours	2
Stage 2	Cervical dilation 5-10 cm	
	0-2 hours	0
	2-4 hours	1
	4-6 hours	2
Stage 3	Expulsive stage to actual delivery	
	0-2 hours	0
	2-4 hours	1
	4-6 hours	2
Stage 4	Delivery	
	Normal attendance	0
	Episiotomy	1
	Low vacuum extraction	2
	High vacuum extraction	3
	Perineal damage to the anal sphincter	2
	Perineal damage through the anal sphincter	3
	Retention of the placenta	1
Stage 5	Postpartum care	
	Normal attendance	0
	Perineal repair	1

Maximal score 17. Easy labor has a score of zero

## RESULTS

A multiple regression model was used to elucidate the main question. By comparing the variance explained by the full regression model including variables for main effects and interactions with a reduced model i.e. the full model minus terms for interaction between the score of the Eysenck Personality Inventory variable and the method of analgesia it was possible to obtain evidence concerning such an interaction.

An overall comparison of the Subjectively Evaluated Pain Suffering (SES) scores after nitrous oxide analgesia and methoxyflurane nitrous oxide analgesia respectively with the difficulty of labour and pudendal block kept constant showed significantly lower SES scores after methoxyflurane exposure (Table II) indicating lower pain suffering after methoxyflurane nitrous oxide analgesia than after nitrous oxide analgesia alone.

Testing for interaction between Eysenck Personality Inventory variables and the method of analgesia gave the following results.

Table II Mean Subjectively Evaluated Suffering (SES) points in a factorial subdivision and comparison between the inhalation analgesia methods showing significantly lower points ( $p < 0.05$ ) after methoxyflurane exposure

	Nitrous oxide	Methoxyflurane
Labor difficulty $\leq 1$	61.4	57.6
No pudendal block	(7)	(21)
Labor difficulty $> 1$	56.9	50.0
No pudendal block	(21)	(16)
Labor difficulty $\leq 1$	63.3	52.8
Pudendal block	(12)	(23)
Labor difficulty $> 1$	72.7	56.9
Pudendal block	(9)	(24)
Weighted mean	63.5	54.0

**Extroversion** The full regression model explained significantly more variance than the reduced model ( $p < 0.01$ ) indicating a significant interaction between extroversion scores and the type of analgesia. It was indicated that the differences between the two analgesia with respect to SES scores varied according to extroversion scores. Table III shows that for subjects whose extroversion scores were higher than the control group given nitrous oxide analgesia alone had significantly ( $p < 0.05$ ) lower SES scores than the group given methoxyflurane nitrous oxide analgesia with pudendal block. Table IV shows the comparison between extroversion scores were 5 or less (introverted) group given methoxyflurane nitrous oxide analgesia with or without additional pudendal block and the

Table III SES points after different types of analgesia. Number of observations in parenthesis. The only significant differences found were for nitrous oxide against methoxyflurane with pudendal block ( $p < 0.05$ )

extroversion score  $> 5$

Parity	Nitrous oxide	Nitrous oxide and pudendal block	Methoxyflurane	Methoxyflurane and pudendal block
Low difficulty	25.0 (1)	62.5 (6)	37.5 (2)	55.0 (4)
High difficulty	50.7 (7)	52.5 (2)	62.9 (7)	76.0 (5)
Weighted means	40.9	56.0	53.2	68.0

had significantly ( $p < 0.05$ ) lower SES scores than the group given nitrous oxide analgesia with or without pudendal block anaesthesia.

Analysis of the effect of the type of analgesia on parity, difficulty of labour and type of inhalation analgesia were kept constant showed that the extroverted parturient tended to have a lower but not statistically significantly different SES score (Table V) when pudendal block was not given. This tendency was not found in the introverted parturient group.

There were no significant differences in mean SES scores between parturients with low and high extroversion scores respectively (Table VI).

Personality and lie scales. The inclusion of neuroticism scores and lie scores in the multiple regression models did not significantly increase the portion of explained variance which indicated that these variables did not influence the SES scores particularly in the introverted group. Thus could not interact significantly with the type of analgesia.

## DISCUSSION

This study was set up primarily to compare the toxicological properties of methoxyflurane with those of nitrous oxide on midwives exposed in alternating

three week periods. However the experiment could also provide data for an evaluation of the difference in effectiveness between the two gases. It would have been preferable to have assigned each parturient individually and randomly to one of the treatment groups. Unfortunately such a design is impossible in a toxicologic study on midwives. However the drawbacks of the three week design were not considered to be of sufficient importance to motivate a separate study of the analgesic effect.

In the puerperal ward the parturient was nursed routinely and met the test leader on the second post partum day. The test leader did not know the type of analgesia the parturient had received nor did he make any remarks, comments or evaluation of his own. Thus he ought not to have influenced the results in any systematic manner.

The Personality Inventory test by Eysenck and Eysenck (7) has been used in other pain studies (4, 12). It is employed routinely at the Psychiatric Clinic in our hospital. The visual analogue scale used for Subjectively Evaluated Pain Suffering (SES scores) was strongly recommended by Hayes and Patterson (10), Freud (9), Aitken (1), Ohnhaus and Adler (15), Scott and Kuskison (16). It has been used in pain as-

Table IV SES points after different types of analgesia. Number of observations in parentheses. Methoxyflurane with or without pudendal block had significantly lower SES points than nitrous oxide and pudendal block ( $p < 0.01$ )

extroversion score  $\leq 5$

Parity	Nitrous oxide	Nitrous oxide and pudendal block	Methoxyflurane	Methoxyflurane and pudendal block
Low difficulty	67.5 (6)	64.2 (6)	59.7 (19)	52.4 (19)
High difficulty	60.0 (14)	78.6 (7)	40.0 (9)	51.8 (19)
Weighted means	63.8	71.3	50.0	52.0

Table V

	No pudendal block added	Pudendal block added
<b>Extroverts (extroversion &gt;5)</b>		
Nitrous oxide	25.0	62.5
Labor diff ≤1	1	6
Nitrous oxide	50.7	52.5
Labor diff >1	7	2
Methoxyflurane	37.5	55.0
Labor diff ≤1	2	4
Methoxyflurane	62.9	76.0
Labor diff >1	7	5
Weighted means	47.4	63.3
<b>Introverts (extroversion scores ≤5)</b>		
Nitrous oxide	67.5	64.2
Labor ≤1	6	11
Nitrous oxide	60.0	78.6
Labor >1	14	7
Methoxyflurane	59.2	52.4
Labor ≤1	19	19
Methoxyflurane	40.0	51.8
Labor >1	9	19
Weighted means	55.2	59.2

assessment in other studies (3, 4). The retrospective assessment of the difficulty of the labor was crude, but although it could not grade the severity of difficult labor, it separated the uneventful labors from the others.

As the Subjectively Evaluated Pain Suffering scores after methoxyflurane analgesia were lower than after nitrous oxide analgesia (Table II, difficulty with labor and the use of pudendal block kept constant), one might have concluded that methoxyflurane was a better analgesic in general than nitrous oxide. However, subdivision according to the extroversion variable of the personality inventory test showed otherwise. In a smaller group of parturients at the extroversion pole, nitrous oxide analgesia gave lower SES scores than methoxyflurane/nitrous oxide. In the larger group of parturients at the introvert pole of the scale, methoxyflurane with or without pudendal block anesthesia gave lower SES scores than the corresponding nitrous oxide analgesia groups. Because introverts were more numerous, the results in that group dictated the overall results.

We could not find any significant relationship between neuroticism and the observed SES scores. This seems to be in agreement with findings reported by Eysenck (8). She stated that neuroticism played no part in predicting either behaviour or attitude to labor of the mother. Bond and Pearson (4) have

shown in women with advanced cervical cancer that the presence of symptoms was related to neuroticism/personality factor, whereas the extroversion dimension of personality was of prime importance in determining the freedom with which these women were communicated. In the present study we found a significant interaction between the extroversion factor and the type of analgesia. Eysenck (8) reported that the extroverts rated their pain in labor greater than the introverts and that this reflected a tendency for them to exaggerate the painfulness of the situation. In this study we could not find any significant difference between low and high extroversion, which might be due to differences in the registration of subjectively evaluated pain suffering. Eysenck (8) used a verbal registration technique and we used a visual analogue scale technique. In a comparative study, Ohnhaus and Adler (15) the visual analogue scale technique proved more reliable than the verbal registration technique.

In the extrovert group of parturients, nitrous oxide analgesia gave lower SES scores than methoxyflurane/nitrous oxide analgesia. In the evaluation of this difference, the tendency of extroverts not to approve pudendal block must be considered (Table VI). We have no explanation for this finding, but the present study indicates that an overall effective analgesia can be obtained when choosing between the various methods by taking into account the patient's psychological make-up.

These findings indicate the possible value of further studies on differences in analgesic effects with respect to personality testing.

Table VI

	Introverts ≤5	Extroverts >5
<b>Nitrous oxide</b>		
Labor ≤1	67.5 (6)	25 (1)
Labor >1	60.0 (14)	50.7 (1)
<b>Pudendal block</b>		
Labor ≤1	64.2 (6)	62.5 (1)
Labor >1	78.6 (7)	52.5 (1)
<b>Methoxyflurane in nitrous oxide</b>		
Labor ≤1	59.7 (19)	37.5 (1)
Labor >1	40.0 (9)	62.9 (1)
<b>Pudendal block</b>		
Labor ≤1	52.4 (19)	55.0 (1)
Labor >1	51.84 (19)	60 (1)
Weighted means	56.8	59.2

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Submitted for publication July 27 1978

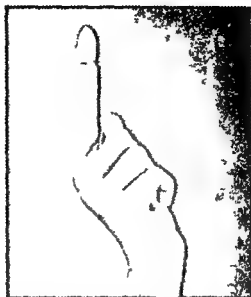
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## APPENDIX

ables for the regression model

- 0 if standard score for personality inventory parameter was less than or equal to 5 ( $\leq 5$ )  
1 if higher than 5 ( $> 5$ )
- 0 if medically assessed labor difficulty was less than or equal to 1 ( $\leq 1$ )  
1 if higher than 1 ( $> 1$ )
- 0 if the parturient was exposed to nitrous oxide  
1 if the parturient was exposed to methoxyflurane
- 0 if the parturient received a successful pudendal block  
1 if the parturient did not receive a pudendal block

- $= x_1$   $x$
- $= x_1$   $x_3$
- $= x_1$   $x_4$  two-way interactions
- $= x$   $x_3$
- $= x_2$   $x_4$
- $= x_3$   $x_4$
- $= x_1$   $x$   $x_3$
- $= x_1$   $x$   $x_4$  three way interactions
- $= x_1$   $x_3$   $x_4$
- $= x$   $x_3$   $x_4$
- $= x_1$   $x_2$   $x_3$   $x_4$  four way interactions

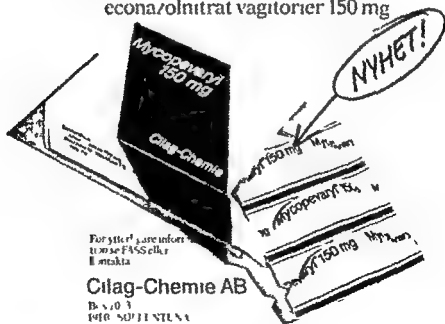


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## TREATMENT OF LABOR PAIN WITH LOCALLY APPLIED KETOCAINE

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**Abstract** Ketocaine a new local anesthetic drug was used for treatment of referred pain during labor. In a randomized double-blind manner fifty primigravidae received compresses containing either ketocaine in a 10 per cent ethanol solution or compresses with saline (placebo). The compresses were applied to the skin areas where the patient experienced the most intense pain. Nineteen of the 25 patients receiving ketocaine compresses reported good or moderate pain relief for an average 5 h (range 1-5 h). These patients had a mean cervical dilation of 3 cm (range 2-5 cm). In patients without effect of the compresses the cervix was dilated to a mean of 6 cm (range 5-8 cm). In patients reporting good effect of the treatment pains were located mainly to the back. Only six of the 25 patients receiving placebo compresses obtained relief of pain. The effect ceased immediately upon removal of the compresses. There was no relation between relief and the degree of cervical dilatation or localization of the pain. No maternal or fetal side effects related to the treatment were registered.

In Scandinavian countries narcotic analgesics sedatives tranquilizers and inhalation analgesics have provided the basis for obstetric analgesia for many years. In effective doses these drugs often cause side-effects both in the mother and in the child. The widespread blockades may also produce side-effects and because of possible risks for the fetus paracervical blockade has been recommended to be performed only by an experienced obstetrician (4). Epidural blockade provides complete relief of labor pain with few side-effects in mother and child. However this extensive method may retard labor and if potent oxytocics then have to be used the combination of analgesia and oxytocics was claimed to increase the risk of fetal distress (8). Furthermore in the very early stage of labor an epidural blockade may be more drastic than necessary. Labor pain is considered to be at its maximum at a cervical dilatation of 8 to 10 cm but it has been shown that even during the early phase of cervical

dilatation some patients especially primigravidae may experience intense pain (3). There is reason to believe that delivery can be delayed in mothers who are stressed because of pain also in the early stage of labor. Therefore if during this period an alternative analgesic less dramatic and more safe than those previously used was available it would imply an obstetric advantage.

Already in 1929 Rose reported that pain during the first stage of labor referred to the lower abdomen and back could be abolished by infiltration of the skin areas with epicutaneous procaine (7). This finding was confirmed by Abrahamson 1950 (1).

It was recently shown (5, 6) that ketocaine (AB Astra Sweden) a new local anesthetic locally applied on intact skin gives a deep-penetrating local anesthesia. The aim of the present investigation was to establish whether pain during labor referred to skin areas above the symphysis and/or sacrum could be decreased or abolished by local application of ketocaine.

## MATERIAL AND METHODS

Fifty primigravidae were included in the study. In a randomized double blind manner they received compresses containing either ketocaine in 10 per cent ethanol solution or saline (placebo). Each compress was delivered in an airtight envelope of plastic-coated aluminum foil. It measured 10 x 14 cm and those with active substance contained 1 g ketocaine (10 ml of a 10 per cent solution).

As can be seen from Table 1 patients receiving ketocaine were comparable with those receiving placebo with respect to age gestational age and degree of cervical dilatation at the application of the compresses.

To an additional 6 patients in whom the absorption of ketocaine was studied compresses containing the drug were applied. In these patients blood samples were taken before application of the compresses and at delivery. Furthermore at delivery blood samples from the umbilical cord or the fetus were analyzed for the amount of ketocaine. The ketocaine analyses were performed at the research laboratories of Astra AB Södertälje Sweden using a mass-fragmentographic method.



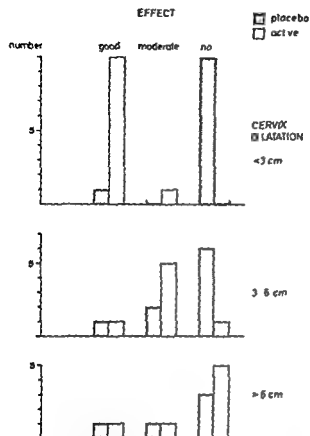


Fig 1 Effect of ketocaine or placebo compresses related to degree of cervical dilatation

**Procedure** The investigation was carried out in the delivery ward. Before and after application of a compress the cervical dilatation was determined by manual palpation. Every patient was observed for one hour.

The woman was requested to indicate on the skin where she experienced the most intense pain. One or two compresses were applied to this area of the skin. If pain was experienced on both abdomen and back, compresses were placed at both sites.

The compresses were left in position for one hour. Then they were removed and the patient's assessment of the effect of treatment was asked for using a simple scale: 0=no effect, X=moderate effect and XX=good effect. One, two and three hours later a new assessment was made. Local effects on the skin were registered.

No analgesics were given during the time of compress application.

The outcome of labor, delivery and the condition of the child were recorded in the routine manner used at the department.

## RESULTS

Of the 25 patients receiving ketocaine compresses 12 reported good effect of the treatment, 7 moderate effect and 6 no effect at all (Fig 1). Patients reporting

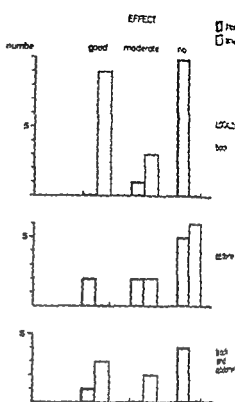


Fig 2 Effect of ketocaine or placebo compresses on localization of pain

good relief had a mean cervical dilatation of (range 2–5 cm). At removal of the compresses 12 patients reporting no effect: the mean cervical dilatation was 6 cm (range 3–8 cm) (Fig 1). It was observed that among patients who reported good relief of pain, 9 out of 12 had their pains localized mainly in the back (Fig 2). The relief of pain lasted on the average for 2.5 hours (range 1–5 hours).

Among the patients receiving placebo compresses 3 reported good effect, 3 moderate relief and 10 no positive effect at all (Fig 1). In no patient did the effect of the treatment persist after removal of the compresses.

In patients receiving placebo the mean cervical dilatation at removal of compresses was 4 cm (range 2–5 cm) (Fig 1). There were no differences in the degree of cervical dilatation between patients reporting good relief and those without relief of pain.

No patient complained of side-effects of treatment. In one woman, however, a marked erythema and edema were found at removal of a ketocaine compress. This local reaction persisted for 3 days but had disappeared when the patient left the department after 7 days.

## Table I Data on the patients

	Ketocaine	Placebo
Age (years)	22 (19-25)	25 (16-32)
Gestational age (weeks)	40 (39-42)	40 (39-42)
Cervical dilatation (cm)	4 (2-8)	4 (1-8)

was generally observed that patients receiving ketocaine more often had erythema and edema of the skin than patients receiving placebo.

No harmful effect on the outcome of labor and delivery could be related to the treatment. All children had 1 minute Apgar scores above 8. Table II gives the maternal and fetal plasma concentrations of ketocaine in the 6 patients receiving ketocaine compresses. As can be seen, the concentrations were low both in mother and in fetus. In no case did the concentration in fetal plasma exceed 25% of that found in maternal plasma.

## DISCUSSION

According to previous studies, pain during the first stage of labor is caused mainly by dilatation of the cervix and to some degree by uterine contractions. (2) The sensory nerves transmitting impulses that produce pain from the uterus enter the spinal cord at the level of the lower thoracic and upper lumbar segments. Mild uterine pain, as experienced during the early part of the first stage of labor, is transmitted through the middle two (T11 and T12) segments. (2) Pain is also referred to the dermatomes supplied by the corresponding segments, i.e. the lower abdominal wall and the skin over the lower lumbar spine and upper sacrum. (2, 3)

The results from the present double blind study show that patients with minor degrees of cervical dilatation and with pain localized to the back obtained the best relief. It seems reasonable to believe that at a cervical dilatation >5 cm, the patient has entered into a state of a stronger pain. Such pain is presumably not only referred, and therefore not possible to treat by application of local anesthetics. This assumption is supported by the observation that in 3 patients with moderate effect of the drug, cervical

dilatation was only 3-4 cm when the compresses were applied, but after 2-3 hours the cervix had dilated to 6 and 7 cm. These patients reported good relief of pain during the first hour, but asked for further analgesics about 1 hour after the removal of the compresses.

To evaluate the effect of ketocaine properly, all patients should have been in a steady state of pain, as well as having a constant degree of cervical dilatation during the investigation. As such conditions are impossible to obtain under clinical circumstances, this must be taken into consideration when evaluating the results.

Doubtless, patients with a minor degree of cervical dilatation, i.e. less than 4 cm, had a good effect of ketocaine compresses compared with those given compresses with saline solution only. Furthermore, patients with placebo compresses noticed that the relief of pain disappeared immediately on removal of the compresses. In contrast, patients with good effect of ketocaine compresses reported that the effect persisted for about 2 hours after removal.

Side effects, except for local erythema in some patients, were not encountered. Measurable amounts of ketocaine were found within one hour in the maternal blood, and the drug did also pass the placenta. However, the measured concentrations were low and probably without clinical significance.

The present results suggest that ketocaine compresses can be used to relieve pain in the early stages of labor. To reveal the mechanism behind the pain relief, and to determine the ultimate place of this therapy in clinical practice, further evaluations are necessary.

Table II Concentrations of ketocaine (ng/ml) in simultaneous samples of maternal and fetal umbilical arterial and venous plasma. Two compresses were applied to each patient.

Case	Maternal vein	Fetal umbilical artery	Fetal umbilical vein	Time after removal of compress (h)
1	61	13	11	3
2	86	15	11	2
3	129	12	31	2
4	93	17	18	4
5	139	30	36	1
6	125	15	17	2

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*Submitted for publication November 27 1981*

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# RELATION OF FETAL HEART RATE PATTERNS WITH UMBILICAL ARTERY pH AND CATECHOLAMINES DURING LAST HOUR OF LABOR

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Fetal heart rate (FHR) patterns were analysed during last hour of labour and correlated to umbilical artery pH and catecholamine (CA) levels. Fetuses with pathological FHR had significantly higher levels of CA than those with normal FHR. Particularly high levels were found in fetuses with bradycardia and late decelerations.

The FHR was further analysed by a computer (DPAC: data processor and computer) in 100 women during last hour of labour. No correlation between deceleration areas or variability and umbilical artery pH and CA was found. It is concluded that although there is a good correlation between the FHR pattern and umbilical artery CA and pH it is not possible to quantify the degree of fetal distress by calculation of deceleration areas and variability.

During the last hour of labor, pathological fetal heart rate (FHR) patterns appear in high frequency. Non-compromized fetuses were found to have decelerations in 25 per cent and compromised fetuses in 50 per cent. The predictive value of FHR patterns during the second stage of labor has been questioned (5, 6, 7). To improve the prediction of fetal asphyxia by cardiotocography (CTG), computer analyses of FHR patterns have been introduced. A significant inverse relationship between deceleration area and both Apgar score and umbilical artery pH has been reported (12) but only partially confirmed (3).

The aim of the present study has been to correlate FHR changes analysed both manually and by a computer with umbilical artery catecholamine (CA) levels and pH. CA levels in umbilical artery have been shown to be a sensitive indicator of fetal distress.

## MATERIAL AND METHODS

The study was undertaken on two groups of patients monitored with cardiotocography (CTG) during the last hour of labor. One group consisted of 89 women, 30 of whom had high risk pregnancies. In this group the FHR patterns were manually evaluated and classified according to Beard *et al.* (2). Ninety per cent of the tracing had to be free from artefacts; otherwise that patient was excluded.

Another group consisted of 100 women, 43 of whom had high risk pregnancies, in which the CTG was linked to a computer unit (DPAC: data processor and computer, Comometrics, Los Angeles, USA). The principal function of this unit has been described (13). All recordings in both groups were performed with scalp electrodes and intra uterine catheters. The following data were processed and printed out by the computer:

1. an average base line calculated during 60 heart beats between uterine contractions
2. long term (LT) fluctuations of the FHR: the sum of amplitude changes in the same direction from beat to beat during a period of 60 heart beats
3. short term (ST) variability: being the short fluctuations of FHR expressed as the sum of amplitude changes in different directions from beat to beat during 60 heart beats
4. total variability: being the sum of LT and ST
5. early deceleration area: calculated 0-40 sec after the start of an uterine contraction defined as a pressure increase of 1 mm Hg/sec for at least 4 sec. Both early and variable decelerations would be included in this recording
6. late deceleration area: calculated 40-80 sec after the start of an uterine contraction
7. total deceleration area: i.e. the sum of early and late deceleration area

Pudendal anesthesia was given to all women. Analgesic drugs were not usually given within 2-3 hours before delivery. A great number of artefacts and FHR-changes were observed during vacuum extractions and these cases were excluded from this study. Forceps were neither used. The umbilical cord was clamped at both ends 15-30 sec after delivery and arterial umbilical blood was aspirated with a syringe. The arterial pH was determined with a blood gas analyser (Radiometer BMS, Copenhagen, Denmark). Plasma was separated and frozen after addition of perchloric acid to a final concentration of 0.4 M. Catecholamines were then isolated from the plasma on alumina columns and determined fluorimetrically as described previously (9).

Neonatal depression was defined as a pH below 7.20 and/or an Apgar score at 1 min. between 0-6.

## RESULTS

In the group of 89 women the different types of FHR patterns were correlated to CA levels and to pH values in the umbilical artery (Table I). Fetuses with pathological FHR patterns showed 3-5 fold higher

Table 1 Apgar scores, umbilical artery catecholamine and pH levels in patients with different types of heart rate patterns

Fetal heart rate pattern	Apgar 1/5	Catecholamines (nmol/l)	t test p-value	pH	t test p-value
Normal (n = 17)	8/10	48.8 ± 9.7	t = 2.43	7.30 ± 0.01	t = 4.93
Bradycardia (n = 7)	5/9	248.5 ± 130.2	p < 0.05	7.17 ± 0.03	p < 0.001
Tachycardia (n = 10)	6/9	205.9 ± 50.9	t = 3.71	7.18 ± 0.04	t = 3.37
Variable decelerations (n = 51)	8/10	106.5 ± 12.4	p < 0.001	7.26 ± 0.01	p < 0.01
Late decelerations (n = 4)	6/10	243.2 ± 198.2	t = 2.38	7.15 ± 0.07	t = 2.09
			p < 0.05		p < 0.05

mean ± SEM, t test against the normal group

levels of CA and significantly decreased pH compared to those with normal FHR. The highest CA levels were seen in fetuses with late decelerations and bradycardia. About 85 per cent of the CA were found to be noradrenaline, the remainder adrenaline.

In Figs 1 and 2 the computer processed data of total variability and late and total deceleration areas were plotted against umbilical artery pH and CA. No correlations between these parameters were observed.

### DISCUSSION

CA are released by the fetus and an increase of the plasma level often seems to precede the development of acidosis (8, 9). In fetal lambs CA injected to produce a similar concentration as versus during hypoxia changes the FHR considerably (8). Increased CA levels have been found in human fetuses with pathological FHR (8) and this is confirmed in this study. Particularly high levels of CA were seen in the fetuses with base line changes. Base line changes have also been observed in sheep fetuses injected with CA in physiological concentrations (1, 4). Whether bradycardia or tachycardia will develop depends on the activation of vagal reflexes and to what extent the CA can overcome those. The fetuses in our series with variable decelerations had higher CA levels than normal but a slightly but significantly lower pH.

This suggests that the occurrence of variable decelerations is a sign of fetal distress, even if the infants have normal Apgar scores.

In this study no correlation was observed between computer analysed deceleration areas (late or total), base line or variability during the last hour of labor and Apgar score at 1 min or umbilical artery pH. These findings are in contrast to some earlier studies.

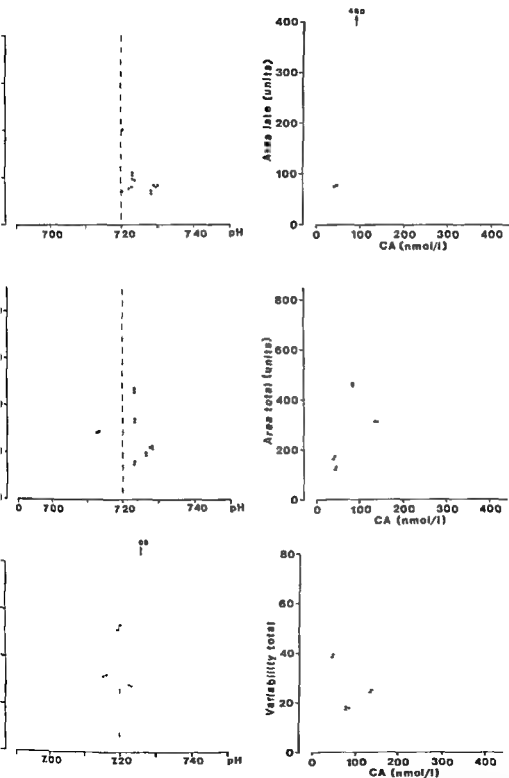
Shelley and Tipton (12) reported that the deceleration area correlated with Apgar scores, but that the mean umbilical pH decreased with increasing deceleration area. However, the correlation coefficient was not more than 0.62. Hon's group (13) reported a reasonable correlation between various deceleration areas, last hour of labor and Apgar scores at 1 min or umbilical artery pH. The correlation coefficient, however, not higher than 0.4–0.6 with higher values for late deceleration areas. Similar results were obtained in their later report (3) when deceleration areas and fetal scalp pH were compared. Coefficients between 0.17–0.45 were found. When also included base line levels and variability in calculations they found that no single characteristic of the FHR would be an accurate predictor of fetal scalp blood pH (11).

Even though we found no correlation between variability or deceleration areas and pH, the fetuses with pathological FHR patterns showed significantly decreased pH values (Table 1). The same was true for correlations with CA.

In conclusion, increased CA levels and decreased pH were observed in all groups with abnormal FHR patterns. However, it seems to be impossible to identify fetal distress from the degree of deceleration or variability, because very subtle changes can lead to fetal asphyxia.

### ACKNOWLEDGEMENTS

This study was supported by grants from Ervén's Odd Fellow's Prenatalforskningsfond, Ålder's Stiftelse, Karolinska Institutet, Allmänna BB and the Swedish Medical Research Council (B78-19X-05-34-01).



Computer processed total variability late and total deceleration areas during last hour of labor plotted against umbilical artery pH

Fig 2 Computer processed total variability late and total deceleration areas during last hour of labor plotted against umbilical artery catecholamine levels

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*Submitted for publication August 4 1978*

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## CONTINUOUS FETAL SCALP TISSUE pH MONITORING DURING LABOR

An analysis of 152 consecutive cases

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## MATERIAL AND METHODS

**Abstract** Continuous scalp tissue pH monitoring was performed in 152 consecutive cases. The success rate was 53 per cent. The average duration of pH monitoring was 151 minutes (range 0-609 minutes). A good correlation was found between tissue pH at delivery and blood pH in the umbilical artery ( $r = 0.78$ ) and vein ( $r = 0.67$ ). In no case was normal tissue pH (more than 7.20) and abnormal CTG. The Apgar score less than 8 one minute after delivery. A low tissue pH almost always means that the fetus is not oxygenated, but a low tissue pH reading may be caused by other factors than acidosis of the fetus. Continuous pH monitoring during labor remains a research method.

Although CardioTocoGraphic Monitoring (CTGM) of the fetus during labor has been used for the last 15 years (7) there are still doubts concerning the benefit of CTGM in both high (4) and low (8) risk pregnancies. Supplementing CTGM with intermittent determination of fetal blood pH reduces the number of surgical operations due to CTGM (4), the disadvantage of fetal blood sampling (FBS) is, however, that it provides no information about the pH in the interval(s) between the blood samplings. This disadvantage may be overcome if a pH electrode for continuous monitoring is introduced into the subcutaneous scalp tissue. A miniature pH electrode developed by Stamm *et al.* (13) and produced by Kontron Roche<sup>(K)</sup> has been available since 1976. We have previously described (18, 19) the original pH monitoring technique and the first results of this monitoring (in 60 and 50 cases respectively). Further results in a total of 152 consecutive cases (including the above mentioned) I have evaluated the value of continuous pH monitoring in predicting neonatal Apgar scores (17). The present paper will deal with the handling of the pH electrode and the reliability of the clinical results of continuous pH monitoring in 152 consecutive cases.

Sixty of the 152 patients were monitored as previously described (19). In the rest of the patients the method was modified. Calibration was performed at 37 °C (instead of at room temperature) and the pH was measured by a separate pH meter (PHM73 Radiometer Copenhagen) connected to a cardiotocograph (8030A Hewlett Packard) (formerly Cardiotocograph 540 Kontron Roche was used). The latter modification makes it possible to register pH values between 7.50 and 7.00 on the cardiotocogram whereas formerly only pH values between 7.40 and 7.00 could be registered. Furthermore the PHM73 maintains its calibration when the pH electrode is disconnected from the pH meter and during the periods when the power supply to the pH meter is switched off.

The pH tracing (Fig. 1) is now seen as a dotted line with a pH registration every 10 seconds.

The pH tracings are classified as

- 1) *acceptable* if 1) a constant pH value is obtained shortly after application of the electrode, 2) pH does not change more than 0.05 pH units during vaginal examinations including gentle moving of the electrode, and 3) pH registration is possible until at least 10 minutes before delivery of the fetal head, and
- 2) *not acceptable* if the above mentioned criteria are not fulfilled.

In order to compare the tissue pH value with blood pH a few experiments were made using fetal blood sampling with subsequent pH estimation. A good correlation between tissue and blood pH was found ( $r = 0.90$ ) (19). Furthermore in the acceptable tracings tissue pH taken at the last minute before delivery has been compared to the pH of the blood in the umbilical artery and vein. If tissue pH monitoring was not possible during the last 1-10 minutes of labor an extrapolated value was assumed as extrapolated pH values have been shown to correlate very well with umbilical artery pH ( $r = 0.83$ ) (17).

In this study the obstetrician looking after the patient did not know the tissue pH and no consequent action was taken.

The drift of the electrode was determined after pH monitoring by recalibration in the buffer solutions of pH 7.00 and 7.40 (37 °C) which had also been used for the first calibration. Maximal electrode drift was calculated as the difference between the recalibration and the calibration value (if the drift was not the same in both buffer solutions the highest numerical value was used).



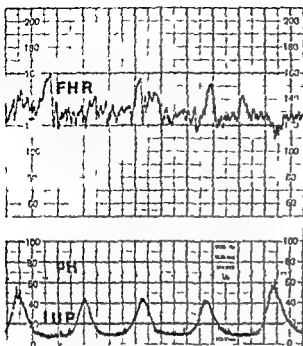


Fig 1 Cardiogram with pH registration (dotted line) 0 mmHg equals pH = 7.00 100 mmHg equals pH = 7.50 The pH is stable between 7.32 and 7.34 Paper speed 1 cm/min FHR = fetal heart rate IUP = intrauterine pressure

## RESULTS

One hundred and fifty-two consecutive pH recordings were made between September 1 1977 and March 1 1979. The majority of women was primiparae (101 = 66 per cent). The pregnancy complications complications during labor neonatal complications and obstetrical operations among the 152 patients are presented in Tables I–IV. Some of the

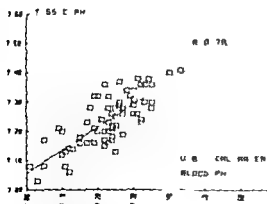


Fig 2 The correlation between the tissue pH at the time of delivery and the umbilical artery blood pH

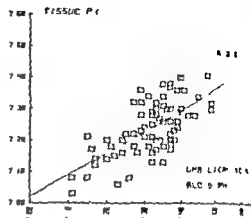


Fig 3 The correlation between the tissue pH at the delivery and the umbilical vein blood pH

results of the first 50 and 60 recordings have been published elsewhere (18–19).

Eighty-one out of 152 recordings (53 per cent) were acceptable. Figs 2 and 3 show the correlation between the tissue pH at delivery and pH of the umbilical artery and vein respectively. The values of 0.78 and 0.67 both correspond to  $p$  values of less than 0.001. There was no change in these relations after modification of the calibration measuring methods (see above).

The drift of the electrode was not correlated with the duration of pH monitoring (Fig 4). The electrode drift in the 134 cases in which recalibration was performed was less than 0.05 in 73 per cent of the cases 0.05–0.09 in 24 per cent of the cases and more than 0.09 pH units in only 2 per cent of the cases. We did not find any change in the drift after changing the calibration temperature from room temperature to 37°C.

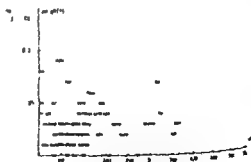


Fig 4 The correlation between the drift of the pH electrode and the duration of pH monitoring. No correlation was found. The correlation coefficient was  $-0.002$ .

PER CENT  
OF CASES

N = 152

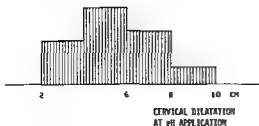


Fig 5 The cervical dilatation at the time of application of H electrode

PER CENT  
OF CASES

N = 152

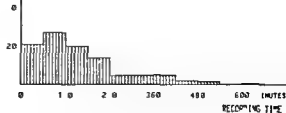


Fig 6 The duration of pH monitoring in the 152 cases

The cervical dilatation at the application of the pH electrode appears in Fig 5

The duration of pH monitoring can be seen in Fig 6. The mean duration was 151 minutes (range 0–609 minutes)

Two cases of scalp abscesses were observed. One of these was treated with incision and antibiotics and the other with antibiotics only. Both infants were born with their mothers at the age of six days. No cases of scalp laceration or bleeding needing treatment were found.

The outcome of the 81 successfully pH monitored deliveries will be published elsewhere (17). It was found that the mean tissue pH in neonates with scores of 7 and 8 one minute after delivery was

7.21 (SEM 0.03) and 7.15 (SEM 0.03) respectively, whereas the mean tissue pH of children receiving higher Apgar scores was 7.25 (SEM 0.01). Two infants had Apgar scores of 0–4 one minute after delivery: one (case 1) after normal CTGM and a pH higher than 7.20; the other (case 2) had a low pH and an abnormal CTG at the time of delivery.

**Case 1** Normal pregnancy and labor. CTGM with pH monitoring for two hours showed a tissue pH of about 7.30 (7.31 at delivery) and 3 late decelerations 0–4 minutes before spontaneous delivery — otherwise the CTGM was normal. The neonate received Apgar scores of 4/10 one/five minutes after delivery. The umbilical artery pH was 7.30. The neonatal period was uneventful and the child was discharged from hospital in good condition at the age of six days.

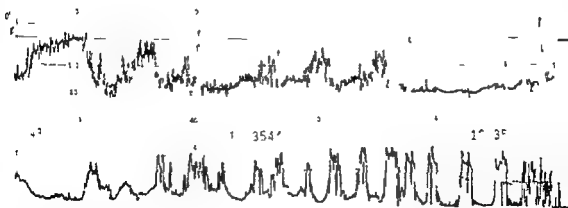


Fig 7 Cardiotocogram with continuous pH registration. The pH decline is indicated by the top of the vertical bars. The tracing was obtained before modification of the method (see above). Paper speed 1 cm/minute. Bradycardia and loss of beat-to-beat variation is seen at the same time as pH declines from 7.36 to 7.21 in 18 minutes.

The pH decline corresponds to a pH decrease of 0.08 units/10 minutes. Umbilical artery blood pH was 7.09. The difference between the tissue and the umbilical artery pH (0.12 units) corresponds to a time lag of 11 minutes. Apgar scores were 9/10 one/five minutes after delivery.

Table I Pregnancy complications

	Number	Per cent
Primipara age $\geq 30$ years	27 (8)	18 (10)
Rhesus immunization	8 (5)	6 (6)
Bleeding before 28th week of gestation	13 (6)	8 (7)
Bleeding after 28th week of gestation	2 (2)	1 (2)
Preeclampsia	8 (5)	5 (6)
Suspected placental insufficiency	8 (5)	4 (6)
Estimated fetal weight $\geq 3600$ g	11 (7)	7 (9)
Postmaturity ( $\geq 42$ th week of gestation)	5 (3)	3 (4)
Other complications <sup>1</sup>	27 (12)	18 (15)
No complications	49 (33)	39 (41)

No. and per cent in brackets refer to acceptable recordings  
<sup>1</sup> One four pregnancy complications as follows: IUD in situ, epileptization before/during pregnancy, obesity, arterial hypertension, premature labor, Caesarean section in earlier pregnancies, appendicitis during pregnancy, urinary infections during pregnancy at birth in earlier pregnancies.

**Case 2** Normal pregnancy except for declining hPL (human placental lactogen) values in the 40th week of gestation leading to induction of labor. CTGM with pH monitoring over the last 40 minutes of labor showed a tissue pH of 7.30 until the second stage. During the second stage, which lasted for ten minutes, the pH declined to 7.18. CTGM was normal until two minutes before the second stage began. At that time the fetal heart rate decreased from 120 to 60 beats per minute and remained at 60 for the remaining 12 minutes of labor. Because of the bradycardia a vacuum extractor was applied during the last five minutes of labor. The child showed Apgar scores of 4/10 one/five minutes after delivery. The umbilical artery pH was 7.15. The neonatal period was uneventful and the child was discharged in good condition at the age of six days.

Table II Complications during labor

	Number	Per cent
Inefficient uterine action	68 (32)	45 (40)
Suspected fetal distress	37 (17)	24 (21)
Meconium stained amniotic fluid	2 (1)	1 (1)
Cephalopelvic disproportion	8 (2)	5 (2)
Maternal exhaustion	3 (1)	2 (1)
Complication after delivery of the child <sup>1</sup>	11 (7)	7 (9)
No complications	48 (29)	32 (36)

Number and per cent in brackets refer to acceptable recordings  
<sup>1</sup> Such as: rupture of the uterine cervix, placental retention, retention of placental cotyledone.

Table III Neonatal complications

	Number	Per cent
Perinatal asphyxia <sup>1</sup>	6 (7)	4 (4)
Apgar score $< 8$ one minute after delivery	11 (6)	1 (1)
Apgar score $< 8$ five minutes after delivery	1 (0)	1 (1)
Hyperbilirubinemia <sup>2</sup>	14 (8)	9 (9)
Weight $< 2501$ g <sup>3</sup>	5 (3)	3 (3)
Perinatal mortality <sup>4</sup>	1 (1)	1 (1)
Scalp abscess	2 (0)	1 (1)
Minor congenital malformations	2 (0)	1 (1)
Down's syndrome	1 (1)	1 (1)
No complications	119 (60)	7 (7)

Number and per cent in brackets refer to acceptable recordings  
<sup>1</sup> Admitted to the intensive neonatal department because of perinatal asphyxia  
<sup>2</sup> One was treated by exchange transfusion, the other by therapy  
<sup>3</sup> No children were born before the 36th week of gestation  
<sup>4</sup> The child died because of cardiac incompetence probably by third trimester intrauterine infection.

In Fig. 2 it is seen that only one infant had an umbilical artery pH of less than 7.17 when the tissue pH was  $\geq 7.20$ . This infant had an umbilical artery pH of 7.13 despite a tissue pH of 7.21. The recording of the delivery is shown in Fig. 7. A possible explanation for this discrepancy between tissue and umbilical artery pH is that the tissue pH was 15 minutes behind the umbilical artery pH as extrapolation of the pH tracing shows that a pH of 7.09 would have been reached 15 minutes before delivery. The Apgar scores of 9/10 one/five minutes after delivery correlated however better with the tissue pH with the umbilical artery pH.

Fig. 2 also illustrates that about 15 per cent of the table tracings showed a low tissue pH (7.13–7.20) with a normal umbilical artery pH (more than 7.20).

Table IV Obstetrical operations

	Number	Per cent
Induction of labor	41 (16)	27 (27)
Stimulation of labor	63 (30)	41 (41)
Epidural analgesia	13 (10)	9 (9)
Low forceps delivery/vacuum extraction	36 (15)	4 (4)
Caesarean section	14 (4)	9 (9)
Operations after delivery <sup>1</sup>	10 (1)	7 (7)
No operations	37 (20)	4 (4)
No operation (stimulation of labor not included)	63 (34)	41 (41)

Number and per cent in brackets refer to acceptable recordings  
<sup>1</sup> Removal of placenta or placental cotyledone manually, ruptured uterine cervix.

Table V Success rate of tissue pH monitoring during labor

Reference	Good quality recordings	Number of trials	Per cent
Allen & Kelly 1978 (1)	16	31	52 <sup>1</sup>
Henner <i>et al.</i> 1978 (2)	44	58	76 <sup>1,2</sup>
Fel & Saling 1978 (3)	7	22	32 <sup>1</sup>
Henner <i>et al.</i> 1978 (4)	35	70	50 <sup>3</sup>
Henner <i>et al.</i> 1978 (5)	14	70	20 <sup>4</sup>
Kubli 1978 (9)	293	447	66 <sup>1,2,6</sup>
Wright <i>et al.</i> 1979 (10)	30	40	75 <sup>1</sup>
Steenegger 1978 (11)	29	43	67 <sup>1</sup>
Bois <i>et al.</i> 1978 1st (14)	30	55	55 <sup>1</sup>
Bois <i>et al.</i> 1978 2nd (14)	56	84	67 <sup>1</sup>
Wright <i>et al.</i> 1978 (15)	47	77	61 <sup>1</sup>
Wright <i>et al.</i> 1978 (15)	33	77	36 <sup>4</sup>
Wright <i>et al.</i> 1978 (21)	43	51	84 <sup>1</sup>
Wright <i>et al.</i> 1978 (19)	31	50	62 <sup>1</sup>
Wright <i>et al.</i> 1978 (19)	18	30	32 <sup>4</sup>
Present material	81	152	53 <sup>3</sup>

Number of unsuccessful second stage recordings not stated/unclassified

Number of unsuccessful applications not stated

Good quality recording = successful recording also during the second stage

Good quality recording = tissue pH recording in which comparison with umbilical artery pH value could be performed

Good quality recording = stable tissue pH recording until at least 15 minutes prior to delivery

Cumulated data from 694 trials in 13 centers

## DISCUSSION

Table I shows the number of good quality recordings in the literature in comparison with the present material. Our investigation shows a lower percentage of successful recordings than most of the other series. This is probably due to the following differences:

We did not classify our recordings as successful if the fetus was not monitored from application of the pH electrode until 0–10 minutes prior to delivery. Only in the materials of Henner *et al.* (5) and Uzan *et al.* (15) was the method of classification similar to ours with approximately the same success rate (20–50 per cent).

Our recording time was more than 120 minutes in 52 per cent of the recordings (Fig. 6) whereas only 29 per cent of the 447 recordings presented by Kubli (9) lasted for more than 120 minutes. Although the drift of the electrode is not correlated with the duration of pH monitoring (Fig. 4) there is a greater risk of incomplete recordings the longer the duration of pH monitoring (18).

The electrode drift in our material is similar to the drift found in other studies (9): more than 0.04 in 18 per cent of the cases. Young *et al.* (22) found that the electrode drift was time related: our data did not support this (Fig. 4). The reason why we did not find the drift to be time related might be that the drift is often most pronounced during the first 15–30 minutes of measurement (*in vitro* observation). This means that the duration of calibration is much more important than the duration of monitoring. In fact, most of our calibrations were performed over long periods as the electrode was often left in the calibration chamber in the morning so only a final calibration was necessary before application of the electrode.

The cervical dilatation at the time of application of the electrode was very similar to ours (Fig. 5) in the cumulated data presented by Kubli (9) (0–4 cm 44.6 per cent, 5–6 cm 32.8 per cent, 7–8 cm 15.0 per cent and 9–10 cm 7.8 per cent).

Although very few cases of tissue pH monitoring in depressed neonates have been published it seems justifiable to use a tissue pH of 7.20 as the limit between normal and pathological pH for three reasons:

- 1 tissue pH in normal fetuses is very similar to scalp blood pH (20)
- 2 if Apgar scores decrease from 9 to 8 one minute after delivery the mean tissue pH decreases from 7.25 to 7.21 (see above) and
- 3 if a tissue pH limit of 7.20 had been used in the management of labor no children would have been born with low Apgar scores because of the tissue pH monitoring (see above).

The very small possibility of a low umbilical artery pH and neonatal Apgar score if tissue pH is normal (see above) justifies our present procedure which does not allow obstetrical operations in cases of pathological CTGM if the tissue pH is normal. On the other hand (Fig. 2) the relatively high number of false low tissue pH recordings found in the present material and by others (12) does not allow obstetrical operations based only on a low tissue pH.

Fig. 7 illustrates that there may be a time lag of 15 minutes between the central arterial pH and the tissue pH. This is in accordance with the data reported by Hochberg *et al.* (6) in the cat. Furthermore it illustrates that acidosis for a short period (in this case 15 minutes) does not influence the neonatal outcome as judged by the Apgar score. The time lag of 15 minutes should therefore not limit the usefulness of tissue pH monitoring.

In another paper (17) I have calculated the sensitivity and specificity of continuous pH monitoring in predicting a neonatal Apgar score one minute after delivery (Apgar score limit between 8 and 9 pH limit between 7.20 and 7.21). I found the sensitivity (67 per cent) and the specificity (73 per cent) of tissue pH monitoring equal to the sensitivity and specificity of cardiotography (50–80 per cent and 50–90 per cent respectively) and discontinuous pH measurements on fetal scalp blood (50–70 per cent and 80–90 per cent respectively) found in a survey of the literature (16).

*The advantages of continuous pH monitoring are*

- 1 Good quality pH recordings are very easy to interpret. With a limit of e.g. pH = 7.20 everybody can tell when the pH is pathological. This is in contrast to the difficult interpretation of a cardiotocogram which may initiate different actions when judged by different obstetricians.
- 2 A number of unnecessary obstetrical operations can be avoided when using continuous pH monitoring. (I estimate the number in this series to be 6/81 deliveries (17)).
- 3 The fetus may be asphyctic in some cases with normal CTGM. In the paper previously mentioned (17) 2/6 infants with an Apgar score of 0–7 one minute after delivery would have been detected by a low tissue pH although the CTGM was normal.
- 4 Only one incision is needed in continuous pH monitoring.

*The disadvantages of continuous pH monitoring with the present electrode are*

- 1 The high number of cases in which pH monitoring cannot be performed during the second stage of labor. As many vacuum extractions and forceps deliveries are caused by ominous CTG patterns a reliable accurate tissue pH recording during the second stage is very important.
- 2 The cases of false low pH readings (see above) mean that obstetrical intervention should not at present be performed because of a low pH reading if it is not followed by a pathological CTGM and/or confirmed by a low capillary blood pH. This also means that although the number of obstetrical operations for suspected fetal distress can be reduced by tissue pH monitoring the cases of false low pH readings will have to be eliminated before all fetuses with a normal pH are to escape an operative delivery for suspected fetal distress.

- 3 To prepare the electrode for application, consuming and the application itself by specially trained obstetrician/midwife.

## CONCLUSION

A pH reading of more than 7.2 has also been found to be associated with a high capillary umbilical artery blood pH (more than 7.15 except one case mentioned above) (Fig. 2). Therefore cases of pathological CTGM a tissue pH of more than 7.20 may prevent unnecessary obstetrical operations. A tissue pH of less than 7.21 may be a false reading, therefore no action can be taken based only on tissue pH.

At present the pH electrode can only be used for research purposes.

## ACKNOWLEDGEMENT

This work was supported by the Dagmar Marckel.

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*Submitted for publication December 30 1979*

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# CONTROLLED SODIUM BICARBONATE INFUSION AND MATERNAL ACID-BASE BALANCE DURING LABOR

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**Abstract** The effects of a controlled sodium bicarbonate infusion on the acid base balance of primiparas in labor and delivery were studied and compared to a control group of primiparas without S B infusion. Starting at 6 cm cervical dilatation 2 mEq of S B/kg of body weight were infused to patients in the study until cervical dilatation. The dosage given to this group was from a study of base deficit dynamics and space infusion of S B in a control group of patients during labor and delivery.

**Significant variations in pH, base excess, and plasma bicarbonate** were observed beginning  $44 \pm 19$  minutes after S B infusion. These variations in acid base parameters started for 10 minutes following delivery.

While no adverse effects were observed in our patients, the experimental design represents a preliminary approach toward correcting deleterious fetal acid base variations during labor and delivery.

During labor and delivery the mother develops a state of metabolic acidosis (1) as a result of acute hyperlactatemia (2), muscular hyperactivity (3), fear and apprehension (4), and insufficient peripheral tissue perfusion (5).

Maternal metabolic acidosis contributes to the fetal metabolic acidosis which is observed during labor and delivery. Any non invasive attempt to influence the fetal acidosis must be performed via the maternal compartment. Such studies (6-11) using an arbitrary amount of sodium bicarbonate (S B) failed to change significantly the fetal acid base balance.

## MATERIAL AND METHODS

The study comprises two groups of primiparas fulfilling the following conditions: uncomplicated singleton pregnancy, presentation without cephalopelvic disproportion, spontaneous onset of labor at term, no sign of fetal distress, normal progress of labor and spontaneous delivery. The first group comprises 12 patients as a control group and the second group of 10 constitutes the S B study group.

Table I indicates the similarity of these two groups. Fig 1 illustrates the partogram of each group while labor was 69 minutes shorter in the S B study group. This difference is statistically not significant. Both groups were treated identically as follows: on admission they were infused with glucose 5 per cent 2 ml per minute. During labor only wetting of the lips was allowed. If membranes were not ruptured on admission, amniotomy was performed at cervical dilatation of 4 cm and Pethidine 75 mg, Promethazine 25 mg were administered i.v. All the labors were monitored by cardiocyclography. Maternal antecubital vein blood samples were obtained on admission anaerobically with a heparinized syringe without venous stasis, then at cervical dilatation of 4-6-8 cm, at full dilatation, head on pelvic floor and at 10 minutes after delivery. Maternal venous blood was used in preference to capillary or arterial blood as it is easy to obtain. It is known that no significant difference in the pH parameters is found between arterial and venous samples (12). The blood samples were analyzed immediately or within 2 hours while kept at 4°C for pH and  $PCO_2$  by blood gas analyzer (Radiometer Copenhagen ABC 1). The Base excess (B.E.) and plasma bicarbonate were calculated according to the Siggaard Andersen alignment nomogram. The hemoglobin value for calculation was 10 gm per cent (Hemoglobin values in all subjects were within the range 9.7 to 11.2 gm per cent).

Table I Comparison between the study and the control group

	Control group		Study group	
	Mean	SD	Mean	SD
Maternal age (years)	22.3	(3.26)	21.4	(2.50)
Gestational age (weeks)	39 w	(0.88)	40 w	(1.58)
Pat. admitted with ruptured memb. (%)	273 d	(6.2)	280 d	(11.1)
Duration of labor (min)	25		20	
First stage	562	(263)	493	(283)
Fetal weight (g)	511	(256)	438	(270)
Apgar 1 min	3.578	(365)	3.419	(428)
Apgar 2 min	8.75	(1.42)	8.50	(1.96)
Apgar 3 min	9.66	(0.78)	9.30	(1.25)
	10.0	(0.00)	10.0	(0.00)

All the differences are not statistically significant.



Table II Acid base balance of maternal venous blood in 12 patients of the control group (mean  $\pm$  SD)

Cervical dilation (cms)	Maternal blood							1 cm
	1-2	2-3	4	6	8	FD	PF	
pH	7.41 (0.03)	7.44 (0.03)	7.46 (0.03)	7.43 (0.03)	7.4 (0.03)	7.41 (0.03)	7.36 (0.03)	7.4
PCO <sub>2</sub> mm Hg	31.6 (6.10)	33.5 (3.40)	33.3 (7.0)	31.2 (5.1)	29.6 (6.0)	30.5 (6.3)	28.2 (6.8)	31.6
HCO <sub>3</sub> mEq/l	19.3 (4.7)	23.7 (5.3)	21.1 (5.5)	20.5 (4.1)	19.7 (4.5)	19.0 (3.1)	15.0 (3.1)	11.8
BE mEq/l	4.0 (4.3)	0.1 (5.0)	2.4 (4.8)	2.9 (3.7)	3.2 (4.9)	4.4 (7.4)	1.8 (3.1)	11.8

FD = full dilation PF = pelvic floor

In the first group the blood samples were obtained as described and no attempt was made to influence the acid base balance. In the second group a solution of 7.5 per cent S B (Abbot Laboratories) was infused according to the following scheme based on the data obtained from the first group. In this group it was found that the maximal maternal base deficit occurred when the fetal head reached the pelvic floor (Table I) this value being  $-8.6 \pm 3.5$  mEq/L (mean and SD). We attempted to correct the maternal metabolic acidosis by S B infusion in an amount arrived at by application of the known formula taking into account BE and S B space of distribution (S D). For ease of administration BE was assumed to be  $-10$  mEq/L. Furthermore S B space of distribution was estimated at 40 per cent rather than the conventional 50 per cent of total body weight (BW) (13) to minimize the possibility of excessive S B administration. The conventional half correction of metabolic acidosis was used to prevent an iatrogenic metabolic alkalosis (13). Thus the following relationships

$$\text{S B infused} = \frac{\text{S D} \times \text{BE}}{2} = \frac{\text{BW} \times 0.4 \times 10}{2}$$

or Amount of S B infused = BW  $\times$  2 in mEq of S B

The total amount of S B administered was 2 mEq per kg of total body weight. The total amount of S B infused

varied from 103 to 176 mEq according to body weight. Infusions were started at cervical dilation of 6 cm and continued until full dilation. This timing was chosen because the decline in maternal pH and the drop in BE as seen in group I (Figs 2-5) occurred before full cervical dilation. Considering the time needed for the distribution of S B in the body and across the placenta, the infusion of S B started before these changes occurred. Continuous infusion was chosen rather than a bolus administration in order to prevent an accentuated acute metabolic alkalosis and rapid S B excretion in the urine. Student's *t*-test was chosen as the statistical method in this study.

## RESULTS

The results for the acid base values of the control group and S B study group are shown in Figs 1 and 2. Table IV compares the differences in pH, PCO<sub>2</sub>, plasma bicarbonate and BE in the maternal blood of the two groups. Until cervical dilation of 6 cm the differences between the two groups in the mother were not statistically significant. From this point on the two groups differ significantly.

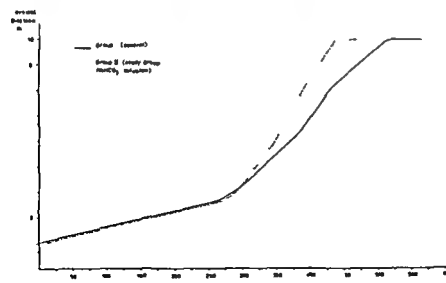
Fig 1 The pH of the maternal blood in the two groups (Mean  $\pm$  SD)

Table III Acid base balance of maternal venous blood in 10 patients of the SB study group (mean and S D)

Cervical dilation (cm)	Maternal blood							10 min after delivery
	1-2	2-3	4	6	8	FD	PF	
pH	7.36 (0.08)	7.41 (0.21)	7.44 (0.40)	7.43 (0.69)	7.51 (0.42)	7.49 (0.51)	7.47 (0.58)	7.43 (0.48)
pO <sub>2</sub> mm Hg	30.2 (8.0)	31.7 (10.4)	27.3 (6.1)	28.3 (7.1)	23.4 (9.0)	29.4 (6.1)	29.4 (7.9)	30.2 (8.8)
pCO <sub>2</sub> mEq/L	16.0 (0)	19.5 (5.5)	18.4 (3.6)	18.4 (4.3)	2.5 (6.3)	23.3 (5.8)	20.9 (5.9)	20.4 (6.3)
mEq/L	8.0 (2.5)	4.0 (5.0)	4.3 (3.1)	4.7 (4.0)	0.2 (5.3)	0.3 (4.8)	1.5 (5.4)	3.0 (6.1)

sodium bicarbonate FD = full dilation PF = pelvic floor

maternal pH mean and (SD) after S B infusion 7.51 (0.42) at cervical dilation of 8 cm compared 7.42 (0.39) in the control group ( $p < 0.001$ ) (Fig. 2). Early significant differences in maternal pH occurred at full dilation and when the head reached the pelvic floor. Ten minutes after delivery maternal pH in both groups was similar (Table IV, Fig. 2) and pO<sub>2</sub> was not significantly different (Table IV, Fig. 3). The differences in plasma bicarbonate (Fig. 4) and (Fig. 5) exhibit similar patterns to those in pH. Maternal BE was significantly higher in the S B group at full dilation than in the control group being +4.8 (4.8) versus -4.4 (2.5) mEq/L ( $p < 0.03$ ). This difference became more pronounced when the head reached the pelvic floor -1.5 (5.4) compared to -8.6 (4.4) mEq/L ( $p < 0.003$ ). No differences in BE per 10 minutes after delivery.

Fetal heart rate during labor and the Apgar score of the infants were normal and similar in the two groups. No dizziness was noticed in the mother serum sodium and potassium remained stable.

## DISCUSSION

In the present work two similar groups of primiparas were chosen. The amount of S B, the time and mode of infusion were based on maternal pH, BE and plasma bicarbonate data obtained in a control group. Likewise, space of distribution and pharmacological dynamics of S B were taken into consideration. In this way the pH of the mother rose 0.09 units ( $p < 0.001$ ) at the stage where cervical dilation was 8 cm. This difference was apparent until delivery. The

Table IV Comparison of acid base balance between the control (Group I) and the study sodium bicarbonate (Group II) (mean and SD)

Cervical dilation (cm)	Maternal blood					10 min after delivery
	6	8	FD	PF		
pH						
I	7.43 (0.43)	7.42 (0.39)	7.41 (0.38)	7.36 (0.40)	7.41 (0.23)	
NS		$p < 0.001$	$p < 0.001$	$p < 0.0001$	NS	
II	7.43 (0.69)	7.51 (0.42)	7.50 (0.51)	7.46 (0.58)	7.44 (0.48)	
pO <sub>2</sub> mm Hg						
I	31 (5.1)	30 (6.0)	30 (6.3)	28 (6.8)	30 (6.0)	
NS		NS	NS	NS	NS	
II	31 (7.1)	31 (9.0)	29 (6.1)	29 (7.9)	30 (8.8)	
pCO <sub>2</sub> mEq/L						
I	20 (4.1)	20 (4.5)	19 (3.2)	15 (3.5)	18 (4.4)	
NS		NS	NS	$p < 0.02$	NS	
II	18 (4.3)	22 (6.3)	23 (5.8)	21 (5.9)	20 (6.3)	
BE mEq/L						
I	2.9 (3.7)	3.2 (4.9)	4.4 (2.4)	8.6 (3.5)	4.9 (4.3)	
NS		NS	$p < 0.03$	$p < 0.003$	NS	
II	4.7 (4.0)	0.2 (5.3)	0.3 (4.8)	1.5 (5.4)	3.0 (6.1)	

NS = no significance FD = full dilation PF = pelvic floor



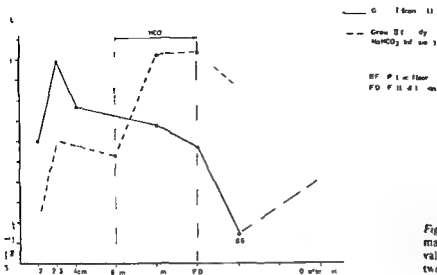


Fig 5 Variation of the maternal Base Excess (mean values) during labor in the two groups

na bicarbonate rose by 6 mEq/L ( $p < 0.02$ ) only when the head was on the pelvic floor. The BE increased at cervical dilation of 8 cm but was first significant at full dilation ( $p < 0.03$ ).

The effect of SB administration on maternal pH was not significantly apparent 44 (SD 19) minutes after the start of the infusion. This time lag probably reflects the distribution time of the SB in the maternal compartment. The time lag was independent of the amount of SB administered.

The effect of SB loading on the mother has to be considered. The administration described here resulted in a mean maternal pH of 7.51 (SD 0.42) at cervical dilation of 8 cm. After this peak, pH dropped to 7.44 (SD 0.48) 10 minutes after delivery. Thus, resultant metabolic alkalosis is temporary and no effects which accompany longstanding metabolic alkalosis were observed.

The beneficial effect of SB on the fetus was found in animal studies. A prolonged survival time was observed in asphyxiated fetal lambs when bicarbonate and glucose were given (14). In asphyxiated fetal lambs treated with bicarbonate and glucose, the incidence and magnitude of brain stem damage were reduced (15). In both these animal studies the results were similar but less pronounced when SB was the sole medication.

These observations justify further studies in humans in order to reduce fetal acidosis in labor and delivery. It seems probable that during fetal asphyxia

the rate of production of acid metabolites is relatively small compared to maternal buffer stores. It would therefore seem logical that in planning such studies the results obtained from the present work in the mother could be taken into consideration as a starting point.

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*Submitted for publication August 14, 1978*

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## TRANSVAGINAL FETOSCOPY IN ANTERIOR PLACENTAS

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**Abstract** When the placenta is located anteriorly fetoscopy by the abdominal route may be unpracticable. An alternative route i.e. insertion of the fetoscope through the anterior vaginal fornix was used in 31 women in midpregnancy. Six of the women had been admitted for therapeutic abortion, the remaining five for diagnosis. The insertion of the instrument was uncomplicated in all 31 women. In three of the five diagnostic cases the disease at risk was excluded. In three women continued their pregnancies and two of them went on to term without complications. The third woman had intermittent leakage of amniotic fluid. At 33 weeks she was delivered of a normal infant (birthweight 3.5 kg). Transvaginal fetoscopy may be considered when the anterior placenta has an extension so broad that the abdominal approach is unfeasible.

If a placenta covers the entire anterior wall of the uterus a fetoscope inserted by the abdominal route may damage the placenta with death of the fetus as a result (1-10). But such a complication can be avoided by insertion of the instrument through the anterior vaginal fornix. We report here our experience with this method.

## PATIENTS AND METHODS

Twenty-one women were selected for this study. They were in the 16th to 24th week of gestation. Ultrasound examination showed the placenta to be located anteriorly. Twenty-six women were admitted for therapeutic abortion, the remaining five for diagnosis. In all cases the woman was in the lithotomy position, the bladder emptied by catheterization. The vulva and vagina were disinfected with an antiseptic solution. The area where the port of entry of the instrument was disinfected with iodine and anesthetized. With the cervix held in position by a tenaculum, a sharp trocar and cannula 2.2 x 2.7 mm in outer diameter were inserted parallel to the cervix into the amniotic cavity. The trocar was removed and an endoscope 1.7 mm in outer diameter (Needlescope, Dyonics, Woburn, Massachusetts) was inserted through the cannula. A 150 mm long endoscope was used in the first cases (5); thereafter a 170 mm long instrument. A large needle was advanced through the channel of the endoscope. Under direct visual control placental vessels were punctured without any attempts at cannula

tion and the stream of blood that issued into the amniotic fluid was aspirated. Usually two or three vessels were punctured in each case.

The proportion between fetal and maternal red cells was determined by the Kleihauer staining for HbF in each sample (8).

Anti-D gammaglobulin was given to rhesus negative women. To prevent uterine contractions a beta receptor stimulator, terbutaline (Bricanyl, Astra Pharmaceuticals) was infused during fetoscopy. In continuing pregnancies terbutaline per os was then prescribed until the end of the 36th week of gestation.

## RESULTS

The insertion of the fetoscope was uncomplicated in all 31 women in the methodology study. There were no signs of damage to the urinary bladder; thus in all women the urine was invariably clear. In one woman who was in the 24th week of gestation the instrument (150 mm long) was too short to reach the placenta. In the remaining 25 women placental vessels were punctured. In one of these women the samples contained only maternal red cells. In three of the women 60, 81 and 85 per cent of the cells were of fetal origin in the sample that had the highest percentage of fetal red cells. In the remaining 21 the corresponding figure was > 95 per cent.

**Diagnostic cases.** Out of five women admitted for prenatal diagnosis the fetus in two was found to have the disease at risk and pregnancy was terminated. In the remaining three who thus had unaffected fetuses two pregnancies went on to term without complications, one woman was delivered at 38 weeks, the other at 40 weeks. The third woman that had an unaffected fetus had undergone amniocentesis twice before fetoscopy, 6 and 3 weeks previously. In this woman the fetoscopy was followed by intermittent

Two fetuses were at risk for hemophilia A, one for hemophilia B (6) and one for von Willebrand's disease (7). In the fifth case fetoscopy was done for chromosomal studies (3).

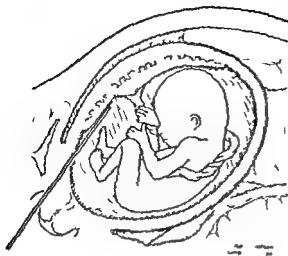


Fig. 1 The fetoscope inserted by the transvaginal route

leakage of amniotic fluid to the vagina. Labor started in the 33rd week. She was delivered of a 2.15 kg normal girl with a 1 minute Apgar score of 9 and a 5 minute score of 10.

No signs of infection were observed in the three women who continued the pregnancy.

### DISCUSSION

According to Elias (4) one of the largest series of fetoscopy all done by the abdominal approach is that of Hobbins and Mahoney at Yale University. In that series 79 pregnancies were allowed to continue after fetoscopy. Five of them ended in spontaneous abortion, in three cases due to amnionitis, in one case to umbilical cord laceration and in one to placental abruption. Seven pregnancies ended in premature delivery. Of the infants thus far delivered none has shown any deleterious effects as a result of the procedure. There has been no maternal morbidity.

In the present study no early complications of fetoscopy by the transvaginal route were seen in any of the 31 women examined. But the number of continuing pregnancies was too small to admit any conclusions about the risk of abortion or premature delivery with this method. We feel that until further experience with transvaginal fetoscopy has been gained the abdominal approach with its well-documented low frequency of abortion and premature delivery is the method of choice in those cases of anterior

placenta where the instrument can be inserted to the placental margin (2, 9, 11). But when the anterior placenta has an extension so broad that abdominal entry is unfeasible the transvaginal approach may be considered. Technically the insertion of the instrument through the anterior vaginal fornix is easy since there is no abdominal wall to penetrate.

### ACKNOWLEDGEMENT

We thank Kerstin Hanneke for technical assistance.

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Submitted for publication July 10, 1980

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# DIAGNOSTIC ULTRASOUND IN THREATENED ABORTION AND SUSPECTED ECTOPIC PREGNANCY

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Of 200 women threatened with abortion during the 6 weeks of pregnancy were examined with diagnostic ultrasound to determine whether there were signs of intrauterine life. Of the 90 who showed positive signs 8 aborted spontaneously later the other 82 continued their pregnancy. Ultrasound investigation revealed no signs of intrauterine life in 110 patients. Of these spontaneous abortion or evacuation because of missed abortion 101 (histopathological examination showed degenerated villi in 98) pregnant 4 mola hydatidosa 3 extrauterine pregnancies.

Ultrasound examination was performed to ascertain if 136 women with suspected ectopic pregnancy had intrauterine pregnancies. 61 of them had an intrauterine gestational sac confirmed at clinical follow up. One of these was operated on with laparoscopy because of pain no malformations were found. In 36 of the other 75 laparoscopy was performed. Ectopic pregnancy 21 ovarian or paratubal cyst 11 adhesions 2 salpingitis 1 and normal 1. Diagnostic ultrasound is excellent for accurate prognosis in threatened abortion. Unnecessary operations avoided. Fertilization in suspected ectopic pregnancies reduced.

Investigation with ultrasound early in pregnancy has been possible only for the past decade. The value of such investigations depends on the certainty with which an intrauterine gestational sac can be seen and whether signs of intrauterine life can be positively demonstrated. Most investigations have demonstrated fetal heart action from week 7 to 8 of pregnancy have shown that a living fetus is a good prognosis for continued pregnancy (1-11). The fetal heart action can be seen on the monitor of a real time scanner or in the A scan of a compound scanner.

Early in pregnancy the gestational sac could be seen as a clearly outlined echo-free space in the uterus on the ultrasound equipment monitor (12). In week 5 of pregnancy the diameter of the echo-free space is 1 cm and the growth is very rapid. A very experienced gynecologist is familiar with the threatened abortion symptoms and the highly fasciculated clinical picture of ectopic pregnancy. Therefore we do not further discuss this here.

## PATIENTS AND METHODS

To evaluate the diagnostic ultrasound in threatened abortion and suspected ectopic pregnancy we investigated for 18 months all pregnancies with these two disorders at the Department of Obstetrics and Gynecology in Lund. The investigations were made either on a real time scanner (Vidoson Siemens) or on a compound scanner (Diasonograph Nuclear Enterprise).

In 200 women with abortion threatened during the first 16 weeks of pregnancy we looked for fetal heart action or fetal movements from week 9 of pregnancy. From week 5 to 8 we measured the diameter of the intrauterine gestational sac according to Hellman *et al.* (12) and determined its growth in two investigations with an interval of at least one week.

In 136 women with suspected ectopic pregnancy we looked for an intrauterine gestational sac.

## RESULTS

Of the 200 women with threatened abortion 90 had signs of intrauterine life 8 of the 90 aborted spontaneously later the other 82 continued their pregnancy.

The ultrasound investigation revealed no signs of intrauterine life in 110 women. 101 of them aborted spontaneously or were evacuated later because of missed abortion (histopathological examination showed degenerated villi in 98) 4 were not pregnant 3 had mola hydatidosa and 2 had ectopic pregnancy (Table I).

Table I Result of ultrasound investigation compared with the outcome of pregnancy

Outcome of pregnancy	Result of ultrasound investigation	
	Life signs	No life signs
Pregnancy continued	82	0
Ectopic pregnancy	0	2
Mola hydatidosa	3	3
Spontaneous abortion	8	101
No pregnancy	0	4
Total	90	110



Table II Result of ultrasound investigations in suspected ectopic pregnancies

Outcome of pregnancy	Result of ultrasound investigation	
	Intrauterine gestational sac	No intrauterine gestational sac
Pregnancy continued	44	1
Therapeutic abortion	9	0
Spontaneous abortion	8	13
No pregnancy	0	40
Ectopic pregnancy	0	21
Total	61	75

Of 136 patients with suspected ectopic pregnancy 61 had an intrauterine gestational sac 44 of the 61 women continued the pregnancy 9 had a therapeutic abortion and 8 aborted spontaneously. None of the 61 had an ectopic pregnancy.

No intrauterine gestational sac could be seen in 75 of the 136 patients.

In fact one of them had an intrauterine pregnancy in week 4 of pregnancy one week later an intrauterine gestational sac could be seen 13 were diagnosed as spontaneous abortions before the ultrasound investigation was performed 40 women were not pregnant 21 had ectopic pregnancy (Table II).

Laparoscopy was performed in 36 of the 75 patients without a visible intrauterine gestational sac on ultrasound (Table III) 21 of them had ectopic pregnancy 11 had ovarian or paraovarian cyst 2 had adhesions 1 had salpingitis and 1 was normal.

One of the 61 women who had a demonstrable intrauterine gestational sac on ultrasound was operated on with laparoscopy for abdominal pains and a normal intrauterine pregnancy was seen.

Of the 21 patients with ectopic pregnancy 16 had positive pregnancy test (HCG in urine) whereas 5 had negative tests. As a comparison 11 of the 13 women with spontaneous abortion had positive pregnancy test.

## DISCUSSION

Women with threatened abortion where no signs of intrauterine life can be noted in one or two ultrasound investigations do not have a living fetus. With modern ultrasound apparatus there is no difficulty in finding an intrauterine pregnancy from the 5-6th week of pregnancy and from the 7-8th week in detecting fetal heartbeats. If signs of life can be demon-

strated the prognosis is very good and the pregnancy has a 90 per cent chance to continue. In threatened abortion ultrasound investigation gives a reasonably safe prognosis and if there is an intrauterine pregnancy without signs of heart activity the embryo can be evacuated thereby avoiding unnecessary loss and pain and reducing the need for hormonal treatment.

Diagnostic ultrasound in cases of suspected pregnancy is most valuable in those patients where an intrauterine gestational sac can be demonstrated. It was possible in 61 of 136 in our material.

In 60 of the 61 patients unnecessary operations were avoided all 60 could leave the hospital within hours after the ultrasound investigation.

In cases of threatened abortion and suspected ectopic pregnancy measurements of HCG are normally useless. Only when ultrasound can demonstrate any intrauterine gestational sac when there is no vaginal bleeding could a pregnancy test be of value and indicate ectopic pregnancy. In several cases recently we have seen the ectopic pregnancy together with an embryo in the uterus with ultrasound. Modern real time apparatus makes the diagnosis more certain and all investigations made with a full urine bladder whereby one can obtain an excellent picture of the entire uterus. There have been any false positive results we have seen where the sometimes thick and partly pedicled endometrium or decidua has been mistaken for an ectopic pregnancy or an abortion in progress. If the results are primarily uncertain the investigation should be repeated in about one week.

Even if the ultrasound demonstration of an intrauterine gestational sac does not exclude the possibility of an ectopic pregnancy with both intra and extra-

Table III Result of ultrasound investigation compared with result of laparoscopy in suspected ectopic pregnancies

Result of laparoscopy	Result of ultrasound investigation	
	Intrauterine gestational sac	No intrauterine gestational sac
Ectopic pregnancy	0	21
Ovarian paraovarian cyst	0	11
Adhesions	0	1
Salpingitis	0	1
Normal	1	1
Total	1	35

uses the possibility of an ectopic pregnancy is very definite symptoms must be present to justify operation. An empty uterus can indicate that the woman is not pregnant, that the pregnancy is so early that it cannot yet be detected by ultrasound, that the woman has already aborted, or that she has an ectopic pregnancy. As mentioned above, an ultrasound investigation in one of our patients was negative in the 13th week of pregnancy, but one week later an intrauterine amniotic sac could be seen. If no intrauterine amniotic sac can be demonstrated, the clinical picture will decide whether operation is necessary. Measurement of human chorionic gonadotrophin in the serum can be useful in these situations.

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Submitted for publication January 23 1979

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# CONTRACEPTIVE HABITS IN WOMEN BETWEEN THIRTY AND FIFTY YEARS OF AGE

A comparison of two periods 1967-69 and 1972-74

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**Abstract** The use of contraception by women 30-50 years of age is presented. The material was composed of part of the prophylactic investigations for cancer of the uterus in the southern part of Storstrøms County which was performed in the period 1967-69 and secondly in 1972-74. A total of 17 078 women were examined of whom 8 234 participated in both surveys. A comparison of two periods of investigation revealed a significant increase ( $p < 0.01$ ) in the utilization of contraception and in turning to more effective methods. This improvement was attributed to the increased amount of information about contraceptive methods which was made available in the country. It was observed that a remarkably large percentage of the women did not use contraception: 24.9 per cent among those 30-34 years of age, 26.9 per cent among those 35-39 years of age, 40.7 per cent among those 40-44 years of age and 57.2 per cent among those 45-49 years of age. Analysis of the influence of socioeconomic conditions showed that women of the lowest social stratum with slight education and low income made up a large proportion of those who abstained from contraception. The number of pregnancies was found to play a role in the use of contraception. An expansion of the distribution of information about contraception to the public is recommended as well as a possible economic subsidy to the socially disadvantaged for procurement of contraceptives.

In recent years considerable progress has been made in the development of methods of contraception. At the same time we have so many different types of effective contraceptives such as combination preparations, single dose preparations, progesterone acting preparations both in tablets and for injection, various types of IUD, diaphragms and condoms that anyone in a position to employ at least one suitable method and obtain satisfactory protection. At the same time dispensation of information about contraception has improved remarkably and has been made easily comprehensible. The mass media in various ways has discussed the concept of contraception. Schools almost everywhere have given instruction in sexuality and guidance in contraception. Contraception clinics are available all over the country

and brochures about contraception are dispensed without charge from hospitals, doctors, midwives etc.

Consequently it should be possible for everyone both men and women to become acquainted with the existing types of contraceptives and to secure adequate protection assuming that pregnancy is not desired.

The main purpose of this work has been to ascertain the contraceptive habits in a section of the population and to detect any changes in these habits after an eight year interval in order to evaluate the influence that the improved development of and information about effective methods of contraception has had on the use of contraception.

A second objective was to determine whether socioeconomic circumstances and the number of pregnancies influence the utilization of contraception.

## MATERIAL AND METHODS

The material was composed of participants in the two prophylactic investigations of cancer of the uterus in women 30-50 years of age carried out in the southern part of Storstrøms county (previously Mønbo county).

The first investigation was performed in the period 1/9 1967 to 1/8 1970 (I) and the second in the period 1/10 1971 to 1/5 1975.

Table 1 shows the patient material. In 1967-69 13 359 women 30-50 years of age were invited to participate in the investigation of whom 13 255 (86.3 per cent) accepted. In 1972-74 14 408 women in the same age group were asked to participate and 12 007 (83.3 per cent) accepted.

Table 1 Patient material

	1967-1969		1972-1974	
	No	per cent	No	per cent
Examined	13 255	86.3	12 007	83.3
Not examined	2 104	13.7	2 401	16.7
Total	15 359	100.0	14 408	100.0

Table II Distribution of different contraceptive methods expressed in percentages in five-year age groups Material 1967-1969

Contraceptive methods	30-34	35-39	40-44	45-49	Total material	
					per cent	%
No	3 0.0	3 3.8	3 4.97	3 3.10		13.2
No contraception	28.7	34.9	46.1	63.9	43.7	5.38
Withdrawal	9.8	13.1	13.2	12.0	12.1	1.62
Other methods	1.8	2.5	2.1	1.6	2.0	0.26
Condom	26.8	25.3	22.9	13.9	21.1	2.73
Diaphragm	7.5	6.1	4.4	2.1	4.9	0.64
Hormonal contraception	41.1	14.5	7.3	3.1	11.2	1.43
IUD	1.7	0.6	0.4	0.1	0.6	0.08
Sterilized	2.6	3.0	3.7	3.4	3.2	0.42
Total	100.0	100.0	100.1	100.1	99.8	

The primary investigation was performed by the 60 general practitioners of the county who in addition to obtaining tissue samples carried out an objective examination of the genitalia, rectum and breasts as well as a relatively detailed gynecological-obstetric anamnesis including questions about contraception utilization (2, 3, 4).

There were a total of 25 262 examinations which included 8 234 women who participated in both surveys.

The data collected were programmed into EDP and have formed the basis of this investigation.

Statistical analysis was carried out by the  $\chi^2$  test at a significance level of 1 per cent. Only  $p$ -values below that level are mentioned. Most of the differences in this work were highly significant with a value of 0.1 per cent. The significant differences in the tables are designated by arrows.

When sterilization is specified for women it indicates all types of gynecological intervention that brought about permanent sterility. These include hysterectomy, salpingectomy etc.

The copper spiral was available for only a short while towards the end of this investigation, namely from 1/1 1974.

The expression 'other methods' includes the rhythm, vaginal douche and foam. Classification by economic groups was carried out according to Sørensen and Wolf (14).

## RESULTS

Tables II and III show the frequency with which various methods of contraception were used. The frequency of failure to use contraception is shown by age groups composed of five-year intervals.

It is immediately evident by comparing the totals that there was a significant decrease in failure to use contraception from 1967-69 to 1972-74. This decrease was accompanied by a significant decline in the employment of withdrawal, other methods and of the condom.

Table III Distribution of different contraceptive methods expressed in percentages in five-year age groups Material 1972-1974

Contraceptive methods	30-34	35-39	40-44	45-49	Total material	
					per cent	%
No	2 9.69	2 9%	2 9.34	3 11.8		1.05
No contraception	4.9	6.9	40.7	47.2	43.6	4.61
Withdrawal	2.6	6.1	4.2	4.0	4.2	0.44
Other methods	0.8	0.4	0.4	0.5	0.5	0.06
Condom	30.1	23.3	18.4	12.5	19.2	2.01
Diaphragm	6.5	6.1	4.0	2.4	4.7	0.49
Hormonal contraception	33.1	6.2	15.8	7.1	20.4	2.14
IUD	5.4	2.8	1.7	0.2	2.5	0.26
Sterilized	3.7	8.1	14.8	16.1	10.8	1.14
Total	100.0	100.0	100.0	100.0	99.9	

↓ = Significant fall compared with 1967-1969.

↑ = Significant increase compared with 1967-1969.

IV *Distribution of contraceptive methods expressed in percentages in different socio-economic groups*  
*erial 1967-1969 Age 30-39 years*

Contraceptive methods	Socio-economic groups					Total material	
	4	5	6	7	8	per cent	No
	205	602	1 696	2 107	1 725		6 335
Contraception	25.9	28.1	28.1	30.5	39.7	31.7	2 074
Withdrawal	2.4	6.5	12.2	12.3	12.8	11.5	731
Other methods	5.4	3.0	2.3	1.9	1.5	2.1	136
Diaphragm	24.9	28.1	27.4	27.0	22.7	26.7	1 645
Condom	16.1	12.1	7.8	7.0	2.6	6.8	4.9
Other contraception	23.9	19.6	19.0	18.3	14.2	17.7	1 119
Sterilization	1.0	2.2	1.4	0.9	0.6	1.1	70
Unprotected	0.5	0.5	1.8	2.1	6.0	2.9	181
Total	100.1	100.1	100.0	100.0	100.1	100.0	

↑ Significant increase between the groups

↓ Significant fall between the groups

the significantly increased use of all contraceptive methods was due to an increase in the most of methods i.e. hormonal contraception IUD utilization. Use of the diaphragm was unchanged

in the individual age groups shows in investigations that failure to use contraception significantly with increasing age though not in the 30-34 to the 35-39 year group in the present survey. In addition the same tendency to a decrease in the use of withdrawal and other methods and of an increase in the use of hormonal contraception IUD and sterilization can be seen in the individual groups as well as in the total material. There are however some exceptions. Condom utilization was unchanged in the 30-34 and in the 45-49 year groups, sterilization

was unchanged in the youngest age group and utilization of IUD was unchanged in the oldest age group. Use of the diaphragm was unchanged in all age groups.

Table IV V VI and VII show the methods of contraception in relation to the classification of the material according to socioeconomic groups. For the sake of clarity the material has been divided into 10-year age groups 30-39 and 40-49 years since no additional information was obtained by arranging the groups in five year intervals.

Comparison of the tables shows that almost the same tendency existed in all the groups in the use or failure to use contraception. In general there was the same significant decrease and increase apart from a few exceptions. With a decline in the level of social status there was a clear indication of the utilization of

V *Distribution of contraceptive methods expressed in percentages in different socio-economic groups*  
*erial 1967-1969 Age 40-49 years*

Contraceptive methods	Socio-economic groups					Total material	
	4	5	6	7	8	per cent	No
	242	602	2 032	1 901	2 096		6 893
Contraception	43.8	50.9	51.4	55.2	59.4	54.6	3 761
Withdrawal	6.6	10.1	13.0	11.0	14.3	11.6	871
Other methods	8.7	2.5	1.7	1.6	1.3	1.9	129
Diaphragm	70.7	19.6	21.3	20.3	13.8	18.6	1 279
Condom	8.7	7.5	3.8	2.8	1.4	3.3	227
Other contraception	8.7	5.9	6.6	5.3	3.3	5.3	36
Sterilization	0.2	0.8	0.2	0.1	0.1	0.2	16
Unprotected	2.7	2.6	2.0	2.8	6.3	3.6	247
Total	100.1	99.9	100.0	100.1	99.9	100.1	

↑ Significant increase between the groups

↓ Significant fall between the groups

Table VI Distribution of contraceptive methods expressed in percentages in different socio-economic material 1972-1974 Age 30-39 years

Contraceptive methods	Socio-economic groups					Total material	
	4	5	6	7	8	per cent	No
No	163	749	1 676	2 030	1 335		5 953
No contraception	17.1	21.9	23.4	24.9	33.9	25.9	1 543
Withdrawal	1.2	2.0	4.4	4.9	5.2	4.3	268
Other methods	1.2	1.1	0.6	0.5	0.4	0.6	36
Condom	15.3	20.4	24.3	26.2	19.4	23.1	1 336
Diaphragm	17.2	12.8	6.6	5.4	2.5	6.3	366
Hormonal contraception	34.4	33.0	31.2	30.3	24.3	29.6	1 661
IUD	9.8	6.3	5.0	3.1	2.7	4.1	241
Sterilized	3.7	2.5	4.5	4.9	11.6	6.0	355
Total	99.9	100.0	100.0	100.2	100.0	99.9	

- = Significant increase between the groups

- = Significant fall between the groups

less effective or no contraception. Highly significant differences in the tables are designated by arrows. A pronounced rise in the number of sterilizations can be seen in the lowest socioeconomic group which was not wholly unexpected.

Table VIII lists the utilization of contraception with regard to the number of pregnancies. This table combines all contraceptive methods (excluding no contraception) since differentiation according to individual methods exhibits interesting relationships only with respect to sterilization. The same inclination was observed in all age groups in both investigations, namely that a large percentage of the women who had never been pregnant abstained from contraception. However, contraception utilization rose steeply after the first and second pregnancies and became stable only after the third pregnancy.

Table IX records the number of surgical sterilizations in relation to the number of pregnancies. It can be seen that a relatively large percentage of women who had never been pregnant were sterilized. A correspondingly high sterilization rate was seen among women who had given birth 4 times. The rate of sterilization among women with 5 pregnancies was not surprisingly quite high, 22.7 per cent among the 40-49 year group in 1973.

## DISCUSSION AND CONCLUSIONS

Our results signify a significant increase in the utilization of contraception during the investigation period 1967-75 and show that the increase was especially due to the employment of safer methods.

Table VII Distribution of contraceptive methods expressed in percentages in different socio-economic material 1972-1974 Age 40-49 years

Contraceptive methods	Socio-economic groups					Total material	
	4	5	6	7	8	per cent	No
No	210	475	1 794	1 876	1 749		6 004
No contraception	34.3	51.2	45.7	47.4	55.9	49.2	2 976
Withdrawal	1.9	2.5	4.5	4.7	3.7	4.1	251
Other methods	2.9	0.0	0.8	0.2	0.3	0.5	33
Condom	15.2	12.6	17.8	17.4	11.6	15.4	937
Diaphragm	9.5	4.4	4.5	2.6	1.3	3.2	197
Hormonal contraception	13.8	16.2	11.8	12.3	8.2	11.3	683
IUD	4.8	1.3	1.0	0.9	0.3	0.9	56
Sterilized	17.6	11.8	13.9	14.7	18.7	15.5	919
Total	100.0	100.0	100.0	100.2	100.0	100.1	

- = Significant increase between the groups

- = Significant fall between the groups

VIII Total use of contraception expressed in percentages in relation to the number of pregnancies in 1967-1969 and 1972-1974 Age 30-50 years

Groups	No. of pregnancies						Total no
	0	1	2	3	4	5	
1969							
39 years	16.5	47.4	75.4	78.3	80.2	76.6	6 348
49 years	9.2	31.0	49.7	53.6	52.6	54.7	6 907
1974							
39 years	22.7	46.9	78.7	84.2	81.9	83.8	5 953
49 years	22.8	37.9	49.7	55.6	59.1	63.3	6 044

Significant increase between the number of pregnancies

Contraception IUD and sterilization results are in complete agreement with other studies (5 & 16)

Nevertheless it is surprising that such a large percentage of women still failed to use contraception. In spite of the encouraging increase in its utilization among the women aged 30-39 years there were 14 per cent who abstained from contraception. It is possible that the true percentage is still larger. In this survey was concerned only with women who participated in the cancer prophylactic investigation. The women who did not attend constitute 14 per cent and 17 per cent respectively and from other studies it has been established that the non attending represents a high risk segment of the population with regard to cervical cancer (10-13). More studies of that group consists predominantly of women from the lower socioeconomic stratum. Consequently it is quite probable that that group utilizes less effective types of contraception or none at all.

acquaintance with the Danish statutes on abortion and sterilization is considered to be an important factor in an assessment of the results of this work. During the period of this investigation there occurred

a considerable liberalization of the legislation with respect to these activities. At the beginning of this period abortion was granted only on the basis of clinical indications but by and large observance of this rule was complied with quite liberally. A statute which granted freedom of choice in abortion was introduced in 1970 for all women over 38 years of age and for women with four or more home living children and permission for abortion to all women pregnant less than 12 weeks was established on 1/10 1973. At the same time the regulations for sterilization were also liberalized so that free determination for sterilization was granted to both men and women over 25 years of age on 1/10 1973.

It seems quite probable that this liberalization has influenced the choice of contraceptive methods since with all other factors constant relaxed restrictions on abortion and sterilization might be expected to be accompanied by less caution with respect to contraception. If that expectation were correct an absence of liberalization of the statutes ought to have produced a still greater shift toward more effective means of contraception than was observed.

In our examination of the influence of socioeco-

IX Frequency (in percentage) of sterilization in relation to the number of pregnancies in ten year age groups

Groups	No. of pregnancies							No
	0	1	2	3	4	5	6	
1969								
39 years	2.9	0.8	0.4	1.3	3.6	10.1	17.2	181
49 years	3.4	0.5	0.4	1.7	4.8	11.5	15.5	247
1974								
39 years	6.8	3.4	2.5	1.5	6.9	13.9	29.3	355
49 years	14.7	12.3	10.2	13.6	16.6	26.7	32.1	938
Total								1 721



conomic conditions on the use of contraception we found this factor to have an extraordinarily large effect. As the social level decreased more ineffective forms of contraception were used. There is a highly significant difference between groups 7 and 8. Svåla, Stoga and Wolf (15) state that group 8 is composed of individuals completely deprived of education and with a low income and that they constitute 25 per cent of the population. There can thus be two reasons for the use of less efficient forms of contraception: one being a lack of comprehension of the concept of contraception and the other a lack of economic ability to purchase contraceptives. In Denmark during the investigation period guidance in questions of contraception was dispensed without charge whereas the implements of contraception must still be paid for by the consumer.

Other authors have studied contraceptive habits in women mostly with younger age groups (7, 8, 9, 12, 14) but one investigation (16) concerned the same age groups as in the present work. A common tendency perceived in all of these investigations was the surprisingly large number of women who failed to use contraception of whom a large proportion were women from disadvantaged conditions. The employment of inefficient contraception or its absence varied from 20–66 per cent in these studies.

The fact that so many women in group 8 have been sterilized is remarkable but understandable. Before the implementation of the above mentioned statutes permitting greater freedom of choice in sterilization there was quite liberal position toward gynecological intervention resulting in infertility and this position was particularly pronounced with regard to women of disadvantaged social status. In addition many women in group 8 had given birth to a large number of children and this in itself served as a reason for sterilization. Finally contraception by means of sterilization is cheap and effortless.

The attitude toward contraception by the nulligravida depicted in Tables VIII and IX can perhaps be explained by the following reasoning. It is quite likely that these women who were never able to become pregnant harbour a strong desire to do so and thereby abstain from contraception. It is also possible that these women believe themselves to be infertile due to the absence of earlier pregnancy and thus use no contraception. The fact that so many nulligravida were sterilized should not be interpreted as evidence that sterilization was performed to achieve infertility but rather that they have undergone a gynecological

operation of one type or another not recorded in the investigation which has caused them to become permanently sterile.

The significant decrease in the use of contraception with increasing age can possibly be interpreted as belief in these women that their fertility decreases with ageing or even that they are infertile, and they reject contraception or utilize less effective methods.

This investigation during an 8-year period in agreement with other work has found an increased utilization of contraception and a development towards increased use of more effective methods. This can be ascribed to the expanded dissemination of information about contraception and the appearance of more and newer types of contraceptives. In the older aged individuals who previously abstained from contraception. Unfortunately the increase in utilization was not adequate and this study had detected a deficit since the segment of the population with the greatest need for effective family planning were themselves most poorly against unwanted conception.

In conclusion increased information about contraception and a possible economic subsidy to the socially disadvantaged is recommended as well as continued research and development in more effective means of contraception (11) with better social stability.

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Submitted for publication May 16 1978

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1982

Date	Place	Name	Office
<b>1980</b>			
September 2-3	Barcelona Spain	Congreso Europeo Perinatal	VII Congreso Europeo de Medicina Perinatal Apartado de Correos No. 29015 Barcelona Spain
September 2-5	Barcelona Spain	7th European Congress of Perinatal Medicine	Congress secretariat Apt de Correo 29015 Barcelona Spain
September 2-6	Berlin Germany	6th International Congress of Psychosomatic Obstetrics and Gynecology	Ass Prof Dr M Stauber Fraenk Charlottenburg der FUB Pulsstr. 4 D 1000 Berlin 19 W Germany
September 4-7	Kiawah Island Charleston SC	International Symposium on Carcinoma of the Cervix Biology Etiology & Diagnosis	E S E Hafez M D OB/GYN State University Medical Res Bldg 550 E Canfield Detroit MI 48201
September 15-16	Bologna Italy	First International Symposium on Recent Advances in Prenatal Diagnosis	A C Assistenza Congressi Via Palagi 21-40138 Bologna Italy
September 22-28	Varna Bulgaria	3rd International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Aging	Bulgarian Academy of Sciences, J G Vassileva Popova c/o Dept of Biophysics 113 Sofia Bulgaria
September 24-26	Kiel West Germany	International Symposium on Fertilization and Artificial Insemination	Priv Doz Dr L Meisler Frauenklinik der Universität Kiel Hegewischstrasse 4 D-2300 Kiel 1
Sept Oct 27-5	Oxford England	Endocrinology of Human Infertility New Concepts	Leslie Nies Symposia Manager Ser Symposia 11 Brooks Drive Braintree MA 02184 USA
Sept Oct 29-1	Freiburg Germany	International Congress on Endocrinology of Human Infertility	C Ferrari M D P O Box 991 Milan Italy
Sept Oct 29-3	Boston MA USA	Gynecologic Endocrinology and Infertility	Harvard Medical School T of Continuing Education 255 South Street Boston MA 02115 USA
September 30	London England	Anti androgen therapy for hirsutism	Symposium Secretary Inst of Obstet Gynaecol Queen Charlotte's Maternity Hospital Goldhawk Road London W6 0XG England
October 3-5	New Delhi India	3rd International Seminar on Maternal and Perinatal Mortality Pregnancy Termination and Sterilization	Hon General Secretary The Fed of Obstetric & Gynecological Societies India Purandare Griha 31/c Dr N A Purandare Marg Bombay 400 007 India
Oct Nov 23-3	San Marino Italy	The Gonadotropins Basic Science and Clinical Aspects in Females	Leslie Nies Symposia Manager Ser Symposia 11 Brooks Drive Braintree MA 02184 USA
November 14-17	Madrid Spain	7 Congrès Européen de Médecine Périnatale	Professor de la Fuente Maternidad de la Ciudad Av Generalissimo 177 Madrid M 4
November 18-23	New Orleans LA USA	AAGL Ninth Annual Meeting Clinical Symposium on gynecologic endoscopy	American Association of Gynecol Laparoscopists 11239 South Lake Boulevard Downey California 90241

# STEROID 16 $\alpha$ HYDROXYLASE FROM HUMAN FETAL LIVER INHIBITION BY STEROIDS

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The 16 $\alpha$  hydroxylase system in fetal liver which dehydroepiandrosterone (DHA) or pregnenolone as substrate was apparently inhibited by various endogenous and synthetic steroids. DHA, pregnenolone, their sulfates, androstenediol, androstenediol, estrone, estradiol, 17 $\beta$  ethynylestradiol and chlormadinone acetate. The inhibition constants ( $K_i$ ) towards DHA were as follows: pregnenolone 4  $\mu$ M, DHA sulfate 13  $\mu$ M, pregnenolone sulfate 21  $\mu$ M, androstenediol 16  $\mu$ M, androstenediol 53  $\mu$ M, estrone 32  $\mu$ M, estradiol 17 $\beta$  75  $\mu$ M, ethynylestradiol 22  $\mu$ M and chlormadinone acetate 27  $\mu$ M. The  $K_i$  values towards pregnenolone were: DHA 6.3  $\mu$ M, DHA sulfate 8.3  $\mu$ M, pregnenolone sulfate 3.9  $\mu$ M, androstenediol 8.7  $\mu$ M, androstenediol 16  $\mu$ M, estrone 15.4  $\mu$ M and ethynylestradiol 16.0  $\mu$ M respectively. The reaction products 16 $\alpha$ OH DHA and 16 $\alpha$ OH pregnenolone showed little inhibitory effect upon 16 $\alpha$  hydroxylase.

Regulation of steroid production by steroids has been demonstrated both *in vivo* and *in vitro*. Corticoids administered to the pregnant patient or to the fetus *in utero* are known to suppress the estrogen level in the mother (1, 2). Placental 3 $\beta$  hydroxysteroid dehydrogenase activity towards DHA was inhibited by endogenous steroids such as 4 androstene 3, 17 and progesterone (10). Similarly, 3 sulfatase activity towards DHA sulfate was suppressed by progesterone and estradiol 17 $\beta$  (9). Little information is available about the controlling mechanism of steroid metabolism in the human fetal liver, which has a major portion of 16 $\alpha$  hydroxylated steroids in the fetal-placental compartments. The present study was undertaken to investigate factors which may modify the activity of 16 $\alpha$  hydroxylase in the human fetal liver *in vitro*.

## MATERIALS AND METHODS

Radioactive steroids [4-<sup>14</sup>C] DHA (58  $\mu$  Ci/m mole) and [3-<sup>14</sup>C] pregnenolone (55  $\mu$  Ci/m mole) were purchased from New England Nuclear Corp., Boston, Mass. Before

use they were purified by thin layer chromatography (TLC). Non-radioactive steroids were obtained from Merck (Darmstadt, Germany), Steraloids Inc. (Wilton, N.H., U.S.A.) or Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Cofactors were purchased from Oriental Co. (Tokyo, Japan). Silica gel GF<sub>254</sub> was obtained from E. Merck. All chemicals used were of analytical grade.

**Tissue Preparation.** Liver was obtained from human fetuses which had been aborted at midtrimester of pregnancy for socio-economic reasons. The tissue was dissected out immediately following delivery of the fetus, weighed, minced and homogenized in a loose fitting Teflon glass homogenizer in ice-cold 0.25 M sucrose solution buffered with 0.05 M Tris HCl at pH 7.4. The homogenate was centrifuged at 10 000  $\times$  g for 20 min, and the supernatant was then centrifuged at 105 000  $\times$  g for 60 min. The precipitate was resuspended in 0.25 M sucrose Tris buffer and again centrifuged at 105 000  $\times$  g for 60 min. The precipitate (the microsomal preparation) was stored at -80  $^{\circ}$ C until used. The protein concentration was measured by the method of Lowry *et al.* (5). The 16 $\alpha$  hydroxylase activity was stable under these conditions for at least 6 months.

**Incubation.** The microsomal preparation of the liver was incubated with either [<sup>14</sup>C] DHA or [<sup>14</sup>C] pregnenolone. Each radioactive steroid substrate (0.05  $\mu$  Ci per incubation flask) the various specific activities were prepared by adding non-radioactive substrate 2.0-50.0 n moles of DHA or 0.5-20.0 n moles of pregnenolone) and the steroid inhibitor to be tested were dissolved in a drop of propylene glycol in an incubation flask together with 0.2  $\mu$  moles of NADPH. The microsomal fraction equivalent to 10 mg of liver tissue (0.067 mg protein) was then added to start the incubation; the final volume was brought to one ml with 0.05 M Tris HCl buffer (pH 7.4). Incubations were carried out for 6 min for [<sup>14</sup>C] DHA and for 10 min when [<sup>14</sup>C] pregnenolone was the substrate. The incubation flasks were shaken constantly in a Dubnoff incubator at 37  $^{\circ}$ C under an atmosphere of O<sub>2</sub> (95 per cent) and CO<sub>2</sub> (5 per cent).

Under these conditions a linear relationship between the time of incubation and the rate of 16 $\alpha$  hydroxylated products formed was established for at least 15 minutes. At the end of each incubation period 5 ml of dichloromethane was added to the flask and shaken vigorously to terminate the reaction and to extract steroids.

**Separation, Quantitation and Identification of Metabolites.** The incubation mixture was extracted with a further 5 ml of dichloromethane. Carrier steroids (75  $\mu$ g each) were added



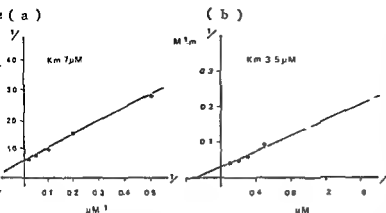


Fig 2 Relationship of the substrate concentrations and the amount of product formed (a) Lineweaver Burk plots for DHA. Increasing concentrations of DHA were incubated with fetal hepatic microsomes (equivalent to 0.067 mg protein per flask) for 6 min (b) Lineweaver Burk plots for pregnenolone. The incubation time was 10 min under the same conditions as in (a)

testosterone ( $p < 0.05$ ) and androstenediol ( $p < 0.05$ ) the apparent  $K_i$  values being 6.3, 8.3, 3.9, 8.7, 4.7  $\mu\text{M}$  respectively.

The reaction products 16 $\alpha$  OH DHA and 16 $\alpha$  OH pregnenolone showed a slight inhibitory effect on 16 $\alpha$  hydroxylation. The  $K_i$  values were 76  $\mu\text{M}$ , 100  $\mu\text{M}$  towards DHA and 45.5  $\mu\text{M}$  and 80.2  $\mu\text{M}$  towards pregnenolone although the effects were not statistically significant.

The other steroids tested only estradiol 17 $\beta$  ( $K_i$  3.2  $\mu\text{M}$  towards DHA and 15.4  $\mu\text{M}$  towards pregnenolone) and ethynylestradiol (22 and 16  $\mu\text{M}$ ) slightly suppressed the 16 $\alpha$  hydroxylase (Fig 3). Progesterone and chlormadinone acetate showed an inhibitory effect only towards DHA ( $K_i$  = 32 and 27  $\mu\text{M}$  respectively). Other steroids had no effect on the 16 $\alpha$  hydroxylation of either substrate.

II Inhibitory effect on 16 $\alpha$  hydroxylation by various steroids. The inhibition constants  $K_i$  are given in Table I for DHA and pregnenolone respectively.

Steroids	Concentration ( $\mu\text{M}$ )	Substrate	
		DHA	Pregnenolone
DHA sulfate	10.0		6.3
Pregnenolone	10.0	11	8.3
Pregnenolone sulfate	10.0	22	
Androstenediol	10.0	21	3.9
Androstenediol	10.0	11	8.7
Androstenediol	10.0	53	14.7
16 OH DHA	50.0	76	45.5
16 OH pregnenolone	50.0	100	80.2
Progesterone	50.0	99	69.0
Progesterone	50.0	32	122.3
Estradiol 17 $\beta$	50.0	75	15.4
Ethynylestradiol	50.0	318	162.0
Progesterone	50.0	22	16.0
Chlormadinone	50.0	131	
Chlormadinone acetate	50.0	91	
Chlormadinone acetate	50.0	27	64.1

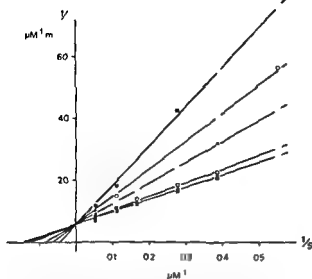
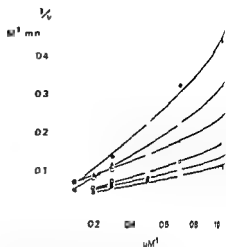


Fig 3 Inhibition of  $16\alpha$  hydroxylation of DHA and pregnenolone by estrogens. The incubation was carried out for 6 min for the substrate  $^{14}\text{C}$  DHA (a) and 10 min for  $^{14}\text{C}$  pregnenolone (b). Inhibitors used:  $\circ$ — $\circ$  estrone,  $\square$ — $\square$  estradiol 17 $\beta$ ,  $\blacksquare$ — $\blacksquare$  ethynylestradiol,  $\triangle$ — $\triangle$  mestranol. They were all added to the media at the concentration of  $50.0 \mu\text{M}$ .



estradiol 17 $\beta$   $\square$ — $\square$  estrone  $\blacksquare$ — $\blacksquare$  ethynylestradiol  $\triangle$ — $\triangle$  mestranol. They were all added to the media at the concentration of  $50.0 \mu\text{M}$ .

The present study deals with the human fetal liver which is not an endocrine organ in the strict sense. However, it is well documented that the fetal liver is the principal site of  $16\alpha$  hydroxylation in human pregnancy (7, 4) and the conditions used in the present work are optimal for detailed studies on the  $16\alpha$  hydroxylation reaction.

Among various steroids added to the incubation media, progesterone had no significant effect on  $16\alpha$  hydroxylase, but androstenediol acted as a competitive inhibitor towards the hydroxylation of both DHA and pregnenolone. It may therefore be postulated that androstenediol plays an important role in the autoregulatory mechanism for the production of  $16\alpha$  hydroxylated steroids in the human fetus. On the other hand,  $16\alpha$  OH DHA and  $16\alpha$  OH pregnenolone, which are the products of the hydroxylation studied, showed less inhibitory effect on the  $16\alpha$  hydroxylation, so that a negative feedback regulatory mechanism by the products does not seem to be demonstrable in the fetal liver.

Estrone, estradiol 17 $\beta$  and ethynylestradiol apparently suppressed the  $16\alpha$  hydroxylase activity. The inhibitory effect of estrone on the formation of  $16\alpha$  OH DHA was more pronounced than that of estradiol 17 $\beta$ . On the other hand, estradiol 17 $\beta$  was a more potent inhibitor than estrone when pregnenolone was the substrate, indicating that the  $16\alpha$  hydroxylase

may have different recognition sites towards estrone and pregnenolone.

The inhibitory effect of ethynylestradiol on hepatic  $16\alpha$  hydroxylase activity was greater than that of the two naturally occurring estrogens, while mestranol was almost ineffective. This provides further support for the assumption that the  $16\alpha$  hydroxylase system in the human fetal liver is furnished with more than one binding site to the enzyme-substrate complexes.

## ACKNOWLEDGEMENTS

We thank Dr T. Akiba, Koga Ibaragi and Dr S. Toki, National Institute of Radiological Sciences, Aomori, Chiba for their help and valuable suggestions in conducting this research.

Thanks are also due to Dr S. Layne, University of Ottawa, Ontario, Canada for his critical review of the manuscript.

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Submitted for publication August 27 1978

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following trivial names are used

dehydroepiandrosterone (DHA)	3 $\beta$ hydroxy 5 androsten 17-one
androstenedione	3 $\beta$ hydroxy 5 pregnen 20-one
androstenediol	5 androstene 3 $\beta$ 17 $\beta$ -diol
androstetriol	5 androstene 3 $\beta$ 16 $\alpha$ 17 $\beta$ triol
estradiol	17 $\alpha$ -ethynyl 1 3 5(10)-estratriene 3 17 $\beta$ -diol
estrone	3 methoxy 17 $\alpha$ -ethynyl 1 3 5(10)-estratriene 17 $\beta$ -ol
estradiol acetate	17 $\alpha$ -ethynyl 17 $\beta$ hydroxy 19-nor-4-androsten 3-one
androstenedione acetate	6-chloro-17 $\alpha$ hydroxy-4 6-pregnadiene 3 20-dione acetate
androstenedione	9 fluoro-11 $\beta$ 17 $\alpha$ 21 trihydroxy 16 $\alpha$ methyl 1 4-pregnadiene 3 20-dione
androstenedione	11 $\beta$ 17 $\alpha$ 21 trihydroxy 1 4-pregnadiene-3,20-dione



# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1982

Date	Place	Name	Office
<b>Continuation</b>			
November 20-22	Barcelona Spain	Symposium International Sobre Monitorizaci3n Prenatal	Instituto Dextus Srias M Luis Arz Ana Baldrich c/Paseo de la 67 Barcelona 17 Spain
November 22-25	Bombay India	First National Congress on Hormones and Human Reproduction	Mahendra N Pankh Organizing Secretary Dr Jhaveri's Hospital Lady Hardinge Road Bombay 40 India
December 1-4	Kairo Egypt	Second Congress of the International Society for the Study of Hypertension in Pregnancy	Docent HJ3rdis Robbe Dept of Obstet and Gynecol karolinska Hospital S-104 01 Stockholm 60 Sweden
December 1-12	Melbourne Australia	Seventh UICC Training Course in Cancer Research	Dr A W Burgess UICC Course The Walter and Eliza Hall Instn Royal Melbourne Hosp P O 304 Victoria Australia
<b>1981</b>			
January 26-31	Mexico City Mexico	Pan American Congress of Andrology	Gerald Bagazinski Congr Admn 31600 West Chicago Livonia, MI USA
March 14-18	Atlanta USA	37th Annual Meeting of the American Fertility Society	The American Fertility Society 1608 13th Avenue South Suite 1 Birmingham Alabama 35204 USA
March 22-26	Berlin West Germany	111rd World Congress of Human Reproduction	Dozent L Mettler Fraenklin Hegewischstr 4 D 2300 Kiel 1
April 15-17	Gorizia Italy	International Course on Ultrasound in Obstetrics	Filippo Destro Organising Secy Dept of Obstetrics and Gynecol City Hospital Via Vittorio Veneto 34170 Gorizia Italy
June 9-12	Ostend Belgium	Third International Congress on the Menopause	The International Menopause Socy 8 av Don Bosco 1150 Brussels
August 24-28	Cambridge England	XIII Acta Endocrinologica Congress	Conference Services Ltd XIII Acta Endocrinologica Congress 3 Bury London SW7 3EY England
Sept-Oct	Athens Greece	V1th European Congress on Sterility (ESCO)	Sekretariat Prof Dr A Seim F Klinik der Universit3t Kiel Hegewisch strasse 4 2300 Kiel 1 West Germany
October 25-31	Melbourne Australia	Eight Asian Congress of Obstetrics and Gynecology	The Organizing Secretary APMA Congress of Obstetrics and Gynecology G P O Box 2195T Melbourne 300 Victoria Australia
<b>1982</b>			
October 17-22	San Francisco CA USA	Xth World Congress of Gynecology and Obstetrics	Xth World Congress of Gynecology Obstetrics c/o The American College Obstetricians and Gynecologists One East Wacker Drive Suite 1700 Chicago Illinois 60601 USA

## EFFECT OF DIHYDROERGOTAMINE ON COAGULATION FACTORS AND COMPONENTS OF THE FIBRINOLYTIC SYSTEM

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Dihydroergotamine (DHE) was given to 20 gynecological patients 0.5 mg i.m. preoperatively and again 12 h and 24 h postoperatively. No cases of thrombosis occurred as judged by the  $^{125}$ I fibrinogen test. Increased concentrations but within normal range were found for fibrinogen, factor VIII activity and related antigen, plasminogen, antithrombin III and decreased concentration for macroglobulin. The fibrinolytic activity of the wall of a superficial hand vein was not suppressed, which otherwise is postoperatively. Together with the increased concentration of antithrombin III this might explain the potential effect of DHE on prophylactic treatment with low-dose heparin.

Use of dihydroergotamine (DHE) combined with low-dose heparin has been reported to reduce the frequency of postoperative venous thrombosis and to be superior to low-dose heparin alone as evaluated by the  $^{125}$ I fibrinogen uptake test (7, 21, 22). Various mechanisms of the effect of DHE have been proposed, such as improvement of the peripheral venous blood flow due to venotonic effects of DHE (10, 11, 20). Sagar *et al.* (21) found that the plasma heparin levels in patients receiving DHE and heparin were significantly higher than in those receiving heparin alone. Kakkar (9) found heparin combined with DHE to produce a pronounced rise in the activity of antithrombin III; this factor is known to be of significance in thrombotic disease (1). The question also arises whether DHE might increase the fibrinolytic activator content of the vein wall, which is an important link in the fibrinolytic defence system and found to be defective in the majority of patients with recurrent thrombosis.

We therefore studied the effect of DHE on the coagulation factors and fibrinolytic components in the wall of a superficial vein of the hand.

## MATERIAL AND METHODS

The study consisted of 20 healthy women with a mean age of 59.7 years (range 49-76 years). All patients had been informed of the aim of the study and gave their full consent. All the patients had been operated on for non-malignant disease or uterine prolapse. The operation was performed under general anesthesia and the blood loss during the operation never exceeded 500 ml, for which reason no blood transfusions were necessary. DHE 0.5 mg was given intramuscularly 3-4 hours preoperatively and then every 12 h for 7 days.

**Diagnosis of deep vein thrombosis.** Each patient received 100  $\mu$ Ci  $^{125}$ I fibrinogen intravenously the day before operation and external scanning with Eberline detector was performed over both legs at 10 different points preoperatively and then 1, 3, 5 and 7 days after the operation.

**Laboratory methods.** Blood samples and venous biopsy specimens were taken preoperatively and on the seventh postoperative day. The following determinations were made:

**Fibrinogen.** The blood was collected with epsilon-aminocaproic acid (EACA) and the fibrinogen was measured using the method of Nilsson & Olow (14). Normal range 0.2 to 0.4 g/100 ml.

**P & P-complex** (prothrombin + factor VIII + factor X) was measured using the method of Owren & Aas (17). Normal range 80 to 120 per cent.

**Factor I levels** were measured using the method of Wolf (24). Normal range 80 to 120 per cent.

**Factor VIII (AHF) activity** was estimated according to Nilsson *et al.* (13). Normal range 60 to 160 per cent. Factor VIII antigen was determined according to Holmberg & Nilsson (5). Normal range 60 to 175 per cent.

**Factor IX levels** were measured using the method of Nilsson *et al.* (15). Normal range 60 to 160 per cent.

**Fibrin/fibrinogen degradation products (FDP)** were measured according to the immunochemical method of Nilsen (12). In the presence of EACA and thrombin this method will not show any FDP in serum from normal patients in whom a level is below 5  $\mu$ g/ml.

**Antithrombin III** was determined immunochemically using the method of Fagerhol & Abildgaard (1). Normal range 60 to 140 per cent.

**Plasminogen** was measured using an immunochemical method (3). Normal range 70 to 130 per cent.

**Inhibitors of urokinase-induced plasminogen activation** (urokinase inhibitors). The clot method was used (19). Normal range 60 to 140 per cent.

# After treatment (arbitrary units)

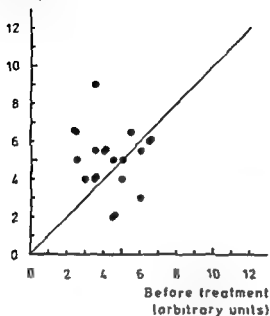


Fig 1 Fibrinolytic activator activity in superficial vein walls before and 7 days after DHE treatment

Alpha<sub>2</sub> macroglobulin was measured with the esterolytic method of Garrot (2). Normal range 80 to 120 per cent. Fibrinolytic activity in the vein wall (18–23). The principle of the method is as follows. Cryostat sections of the specimens collected on glass slides are covered with a plasminogen rich fibrin layer. After certain periods of incubation the slides are fixed and stained. If plasminogen activators are present they will convert plasminogen into plasmin with digestion of the overlying fibrin layer. The digested areas are seen as pale zones in the stained fibrin film. They vary in size with the activator activity and can be graded in arbitrary units. Normal range 6–10 arbitrary units.

Venous biopsy specimens were obtained from the dorsal aspect of the hand under local anaesthesia (Carbocain®) 5 per cent. After a longitudinal incision of the skin had been made a 0.5–1.0 cm segment of the vein was exposed carefully dissected and excised.

**Statistical methods.** The level of significance was calculated by Student's *t* test for paired observations. The difference between the fibrinolytic activator content of biopsy specimens was estimated by Wilcoxon's rank sum test.

## RESULTS

In none of the 20 patients did the <sup>125</sup>I fibrinogen test show any evidence of thrombosis. Two patients with a slightly increased <sup>125</sup>I fibrinogen uptake were also examined using phlebography which similarly showed no signs of thrombosis.

All the mean values for the coagulation factors and components of the fibrinolytic system were within the normal range before and after therapy (Table 1). Significant changes were noted for fibrinogen, factor VIII activity and antigen plasminogen antibody. III and alpha<sub>2</sub> macroglobulin.

The median value of fibrinolytic activator in the vein wall was 4.5 (2.5–6.5) arbitrary units before and (2–9) after treatment, a slight increase which was significant (Fig 1).

## DISCUSSION

The results show that the fibrinolytic activator content in the vein wall was unchanged or even slightly increased. A low fibrinolytic activity in the vessel wall is a common defect in patients with thrombotic disease (8). Åberg & Nilsson (25) found a decrease in this fibrinolytic activity to be a common postoperative finding, especially after major surgery. Our findings therefore indicate that DHE counteracts postoperative suppression of the fibrinolytic system.

In the present study fibrinogen and factor VIII were increased but still lay within the normal range. These changes are in agreement with earlier reports where they were ascribed to surgical trauma (6).

Antithrombin III has received increasing attention in recent years as in some families suffering from severe thrombotic disease low concentrations of antithrombin III have been found (1). Saga et al (1981).

Table 1 Coagulation factors and components of the fibrinolytic system measured before and 7 days after treatment with dihydroergotamine (n = 20)

	Before treatment	After treatment	SD	Significance
Fibrinogen (g/l)	4.1	4.9	0.6	<0.05
Factor V (%)	95	93	9.4	n.s.
P & P (prothrombin time) (s)	106	111	15.8	n.s.
Factor VIII (%)	138	187	4.4	<0.05
Factor XIII (%)	105	160	72.4	<0.05
Factor IX (%)	112	121	24.6	n.s.
Anti-thrombin III (%)	90	97	8.5	<0.05
FDP (µg/ml)	1.2	2.4	2.4	<0.05
Plasminogen (%)	109	125	1.0	<0.05
Urokinase inhibitors (%)	115	122	16.5	n.s.
α <sub>2</sub> macroglobulin (%)	105	97	14.0	<0.05

) mean; †) 7th day mean

onstrated that patients who had preoperative an  
ombin III activity of less than 70 per cent of nor  
had deep venous thrombosis in the postoperative  
d. Low-dose heparin potentiates the activity of  
thrombin III which is the main explanation for  
fect in the prevention of thrombosis. Kakkar (9)  
d heparin combined with DHE to raise the ac  
of antithrombin III substantially more than  
heparin alone. Our finding of an increased con  
ation of antithrombin III following administra  
of DHE suggests a synergistic effect of DHE and  
in on the concentration of antithrombin III.  
The frequency of postoperative venous thrombosis  
iated with the <sup>125</sup>I method has been reported to  
1-18 per cent in a gynecological material (4).  
Test was performed to check coagulation factors  
fibrinolytic components. In this small study the  
ce of thrombotic complications confirms the  
thrombotic effect of DHE which might be ex  
ed by an increased concentration of antithrom  
II and unchanged fibrinolytic activity in the vein

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Submitted for publication November 4 1978

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## ANNOUNCEMENT

**The Vth International Congress of Psychosomatic Obstetrics and Gynecology** will take place in West Berlin September 2-6 1980. The principle theme of the Congress — *Women in a changing society* — is meant to encourage lectures and discussions dealing with socio-psychological characteristics and cultural influences, psychosomatic symptoms and illness as well as with the psychotherapeutic potential in obstetrics and gynecology.

The Congress program includes plenary lectures delivered by invited speakers as well as short lectures on recent psychosomatic findings. In addition to those working groups scientific films and demonstrations are being planned.

### *Languages of the Congress*

English, French, Spanish and German

*Information may be received from*

Scientific Secretariat

Ass. Prof. Dr. M. Stauber

Frauenklinik Charlottenburg der FUB

Pulsstrasse 4

D 1000 Berlin (West) 19

West Germany

Organizational Secretariat

W. Syborg (COG)

John Foster Dulles Allee 10

D 1000 Berlin (West) 21

West Germany

**An International Symposium on Carcinoma of the Cervix** will be held on Kiawah Island, Charleston, South Carolina, USA, September 4-7, 1980.

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**The First International Symposium on Recent Advances in Prenatal Diagnosis** will be held at the Palazzo della Città dei Congressi in Bologna, Italy, on the 14th and 15th September 1980. The symposium, organized by the Gynecological and Gynecological Department of the Bologna University and by the F. Angelini Research Institute, Rome, will include a number of Round Tables on the main aspects of prenatal diagnosis.

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**Xth World Congress on Fertility and Sterility** The International Federation of Fertility Societies will sponsor the World Congress to be hosted by the Spanish Fertility Society at the National Palace of Expositions and Congresses, Madrid, Spain, September 20-26, 1980.

There will be two days for Pre-Congress Symposia, Post-Graduate Courses, followed by a program on the following themes:

- Spermatogenesis
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  - Psychosexual and social aspects of fertility
  - Problems of gamete transportation
- Immunology in reproduction
  - Control of fertility
- Neuroendocrinology of reproduction
- Fertilization and implantation
  - Environmental and iatrogenic aspects of reproduction
  - Genetics in reproduction

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EFFECTS OF ETHINYLESTRADIOL/D-NORGESTREL COMBINATIONS  
ON SERUM LIPOPROTEINS

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tract Serum lipoproteins were analyzed in 33 young women (mean age 22 years) before and after two cycles of treatment with one of the following ethinylestradiol/d norgestrel combinations ( $\mu\text{g}/\mu\text{g}$ ) 30/150 50/150 and 50/250. A slight but significant weight increase of 1.2 kg was found in the 50/150 group. All three hormone combinations had similar effects on the lipoproteins. Before treatment all subjects had normal lipoprotein concentrations. Whole serum triglyceride (TG) and cholesterol concentrations were significantly affected. In the whole group the LDL TG concentration increased from 0.24 to 0.33 mmol/l ( $p < 0.01$ ) and HDL-cholesterol fell from 1.54 to 1.35 mmol/l ( $p < 0.001$ ). Smokers tended to have lower HDL-cholesterol than non-smokers. It is possible that HDL reductions for longer periods of time may be one of the factors that promote the development of atherosclerotic manifestations in young women on oral contraceptives.

Oral contraceptives are often used for several years during which a woman is exposed to the metabolic abnormalities associated with this treatment. However, oral contraceptives of today contain considerably lower concentrations of hormones than some years ago with less unwanted side effects. In 1971 we studied the effects of Anconce<sup>®</sup> (0.1 mg mestrol + 3 mg chlormadinone acetate) on serum lipoproteins (16). After two months the serum triglyceride (TG) concentration had almost doubled and the serum cholesterol concentration increased 12 per cent.

The TG and cholesterol concentrations rose in the very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions. Continuing medication with only chlormadinone acetate led to normalisation of these values but not until after six weeks.

Lynn *et al.* demonstrated less marked serum lipid changes after administration of a low dose preparation containing 30  $\mu\text{g}$  ethinylestradiol + 150  $\mu\text{g}$  d norgestrel (21). The mean serum TG concentration increased by about 10 per cent and serum cholesterol by 10 per cent. Still such changes of whole serum lipoprotein concentrations do not appear unless the lipopro-

tein concentrations of TG and cholesterol have been significantly affected (6). We therefore analysed serum lipoproteins in detail in women on low dose oral contraceptives. Since combinations of estrogen and progestagens may have complex effects on lipoprotein metabolism, three different hormone combinations were chosen.

## SUBJECTS

The women were recruited from a contraceptive clinic run by the Stockholm County Civil County where they all applied for prescriptions of oral contraceptives. The gynecologist who examined them invited them to take part in the study and their informed consent was obtained. The recommendations by the Swedish National Board of Health 1977 were followed for exclusion of women for whom oral contraception were considered not to be advisable. No woman had used oral contraceptives later than 6 months prior to the present study.

Forty-one women (mean age 22 years, range 17-29 years) were referred to the Lipid Unit, Karolinska Hospital, where a fasting blood specimen was drawn for lipoprotein analysis. The body weight was recorded and they were asked about smoking and drinking habits. At random the women were then given one of three contraceptive preparations for two cycles and were asked to return at that time for another blood sample. Both samples were drawn between the 21st and 26th day of the menstrual cycle. The patients were evenly recruited to the study throughout one whole year in order to eliminate the influence of seasonal variation on lipid concentrations. For unknown reasons 8 women who had come to the Lipid Unit for the first visit did not appear on the second occasion in spite of repeated calls.

Three different drugs were used (kindly supplied by AB Recip, Stockholm). They contained ethinylestradiol/d norgestrel in the following combinations: 30  $\mu\text{g}$ /150  $\mu\text{g}$ , 50  $\mu\text{g}$ /150  $\mu\text{g}$  and 50  $\mu\text{g}$ /250  $\mu\text{g}$ .

Serum lipoproteins were determined by preparative ultracentrifugation as described by K. Carlson (5). After centrifugation at  $d = 1.006$  the top fraction was harvested and called the VLDL fraction. Heparin-manganese chloride was added to the infranant which precipitated the B-apoprotein containing LDL fraction. The remaining lipoprotein in solution was defined as the HDL fraction. The concentration of TG and cholesterol in whole serum, VLDL, LDL +

Table I Body weight (kg mean  $\pm$  SEM) before and during oral contraceptive treatment with drugs of various combinations

Horm comp estr/d norg <sup>†</sup>	No	Body weight		Sign diff
		Before	During	
30/150	11	62.8 $\pm$ 2.2	63.1 $\pm$ 2.2	p > 0.05
50/150	9	58.2 $\pm$ 2.2	59.4 $\pm$ 2.1	p < 0.05
50/250	13	64.5 $\pm$ 2.4	64.8 $\pm$ 2.4	p > 0.05

<sup>†</sup>ethinylestradiol/d norgestrel ( $\mu$ g). Student's t-test, paired data

HDL were determined by Technicon Auto Analyzer methods (3, 10). The LDL concentrations of TG and cholesterol were calculated by subtraction. The recovery ranged from 90–110 per cent.

## RESULTS

Thirty three women completed the study. There were no major complaints with regard to drug tolerance. The body weight before and after treatment is shown in Table I. 50 per cent of the women were non smokers whereas 37 per cent smoked more than 10 cigarettes/day. 13 per cent consumed more than one bottle of wine/week (or alcohol in corresponding quantities). The three drug groups were similar with regard to age, smoking and drinking habits.

Before treatment the lipoprotein concentrations were similar in the three groups (Table II). All women had low to normal serum lipid concentrations and no subject developed hyperlipoproteinemia during treatment. The serum concentrations of total TG and cholesterol did not change significantly during treat-

ment with either preparation although there was a tendency for TG to rise. In the VLDL fraction, TG and cholesterol concentrations were not increased by any preparation and the VLDL cholesterol/TG ratio was unaffected. In LDL the TG increased by about 35 per cent in all three groups (p < 0.01). The LDL cholesterol concentration did not change. In the HDL fraction a slight TG increase was observed after treatment only with the 50/150. The HDL cholesterol concentration fell by a value of 12 per cent in all groups (p < 0.001). 50/250 and the 30/150 groups significant differences were found (p < 0.001 and p < 0.05) whereas the reduction found in the smaller group did not quite reach statistical significance. In smokers the mean HDL-cholesterol was 1.44  $\pm$  0.10 mmol/l and for non smokers 1.60  $\pm$  0.08 mmol/l but this trend was not statistically significant. The mean alcohol consumption was modest in this group of women and did not significantly affect the serum lipoproteins.

## DISCUSSION

A review of studies on the effects of steroid contraceptives on lipid metabolism is confusing. Different designs have been different. Data from different types of steroids have been combined. In studies the women have not been their own controls and furthermore the women have been on treatment for periods which have varied from weeks to years. Finally results from studies of postmenopausal women on hormonal replacement therapy are

Table II Triglyceride (TG) and cholesterol (Chol) concentrations (mmol/l mean  $\pm$  SEM) in the lipoprotein fractions before and during treatment with oral contraceptives of varying hormonal composition

Horm comp estr/d norg <sup>†</sup>	VLDL		LDL		HDL		Total
	TG	Chol	TG	Chol	TG	Chol	TG
30/150							
Before	0.43 $\pm$ 0.07	0.23 $\pm$ 0.05	0.23 $\pm$ 0.02	2.73 $\pm$ 0.15	0.13 $\pm$ 0.02	1.68 $\pm$ 0.09	0.83 $\pm$ 0.10
During	0.46 $\pm$ 0.05	0.28 $\pm$ 0.03	0.31 $\pm$ 0.02	2.86 $\pm$ 0.31	0.14 $\pm$ 0.02	1.44 $\pm$ 0.08	0.96 $\pm$ 0.07
50/150							
Before	0.61 $\pm$ 0.12	0.34 $\pm$ 0.05	0.26 $\pm$ 0.03	2.94 $\pm$ 0.10	0.14 $\pm$ 0.03	1.37 $\pm$ 0.10	1.05 $\pm$ 0.13
During	0.61 $\pm$ 0.07	0.36 $\pm$ 0.05	0.35 $\pm$ 0.02	2.92 $\pm$ 0.10	0.17 $\pm$ 0.02	1.26 $\pm$ 0.10	1.17 $\pm$ 0.09
50/250							
Before	0.53 $\pm$ 0.09	0.31 $\pm$ 0.05	0.25 $\pm$ 0.04	2.68 $\pm$ 0.23	0.15 $\pm$ 0.01	1.52 $\pm$ 0.10	0.96 $\pm$ 0.14
During	0.48 $\pm$ 0.06	0.28 $\pm$ 0.03	0.33 $\pm$ 0.03	2.81 $\pm$ 0.16	0.15 $\pm$ 0.01	1.34 $\pm$ 0.08	1.00 $\pm$ 0.09
All women							
Before	0.52 $\pm$ 0.04	0.28 $\pm$ 0.03	0.24 $\pm$ 0.02	2.78 $\pm$ 0.10	0.14 $\pm$ 0.01	1.54 $\pm$ 0.06	0.94 $\pm$ 0.07
During	0.51 $\pm$ 0.04	0.30 $\pm$ 0.03	0.33 $\pm$ 0.01	2.86 $\pm$ 0.12	0.15 $\pm$ 0.01	1.35 $\pm$ 0.05	1.03 $\pm$ 0.05

<sup>†</sup>ethinylestradiol/d norgestrel ( $\mu$ g). Statistically significant differences (Student's paired t test) are indicated. p < 0.05 \*\*\*p < 0.001

other results than studies of young or middle age on oral contraceptives

In our study the women were their own controls. The three hormone combinations could be studied under identical conditions. The follow up period was 12 months. However, a longer time interval would probably have led to a considerable drop out of these young and symptom free women.

In spite of different compositions the three drugs used in this study gave similar results. Total serum cholesterol concentrations did not increase although there was a tendency for serum TG to increase in accordance with other studies (13, 17, 18, 21). In contrast to some of these studies, no significant serum cholesterol reduction was observed (13, 18, 21). It has been suggested that a possible metabolic advantage of these low dose oral contraceptives is the fact that they may increase the serum cholesterol concentration (21). This undoubtedly has been an advantage, had the reduction occurred in a lipoprotein fraction with atherogenic properties. However, in our study the cholesterol concentrations in the atherogenic VLDL and LDL fractions remained unaffected and the only cholesterol reduction was found in HDL. Since there is a general agreement that a low HDL concentration is associated with increased risks for atherosclerotic manifestations it seems unlikely that LDL reduction observed by us is beneficial for women.

Smoking is known to reduce HDL levels and moderate alcohol consumption to increase the HDL concentration (7, 8). Since drinking habits were stable in these 33 young women, no systematic effect of alcohol consumption could be detected. However, even in this small group, smokers had a trend for 1 per cent lower HDL cholesterol levels although the reduction was not quite significant.

The isolated 35 per cent LDL TG increase during therapy is a new and uncommon finding. An inverse relationship between the VLDL TG and the HDL cholesterol concentrations is well known (15). The reduction would therefore rather have been associated with a VLDL TG increase. The significance of the LDL TG increase is difficult to interpret. In the LDL is considered to be the endproduct of VLDL after TG removal from its core and concomitant rearrangement of the surface structures (15). An isolated LDL TG increase would rather suggest an increased clearance of LDL TG, since in this study the LDL concentrations remained unaffected.

Estrogens stimulate hepatic TG secretion (9). Im-

paired lipolysis has also been described and in a recent study selective impairment of the hepatic lipase activity could be demonstrated (1). On the other hand, ethinylestradiol (1 µg/kg body weight and day) caused a paradoxical normalisation of the type III broad beta disease hyperlipoproteinemia which is characterized by the accumulation of abnormal VLDL remnants (12). In that study the LDL lipids were unaffected and both TG and cholesterol concentrations increased in HDL. These estrogen doses were slightly higher than used in our study. It is obvious that effects on the patients with type III hyperlipoproteinemia may differ greatly from the effects on the low serum lipoprotein concentrations seen in healthy young women. It is possible that in our study the estrogenic effects may have been partly counteracted by the norgestrel component of the oral contraceptives.

Krauss *et al.* have compared lipoprotein levels in 18 users with 19 age-matched non users (11). A significant increase was found only of the HDL fraction. By detailed fractionation this increase could be located to the denser part of this fraction, the so-called HDL<sub>2</sub>. However, since the 18 women studied had used oral contraceptives of seven different compositions a strict comparison is difficult to perform.

Several epidemiologic studies have compared serum lipid levels of hormone users to non users and obtained quite different results. The Lipid Research Clinic Program found a 48 per cent increase of the plasma TG concentration in women on oral contraceptives aged 20–24 years (19). The mean plasma cholesterol concentration was 5 per cent higher. However, these investigators never specifically asked for the exact type of hormone medication.

In the Leiden population study the HDL cholesterol concentration was about 30 per cent lower in women on oral contraceptives than in non users (2). It is possible that clinical conformity in the Leiden area has led to prescription of similar low dose preparations which might explain a more uniform pattern of lipoprotein values.

Only in a recent study (Contraceptive Drug Study, Kaiser Foundation Health Plan) the importance of the type of oral contraceptive was taken into account in the lipoprotein data analysis (4). These 4 978 women supplied information about smoking, drinking habits and about the brand and dosage of the hormone preparation they were using. Estrogen users had significantly higher HDL-cholesterol concentrations than controls. In progestin users, significantly



lower HDL cholesterol concentrations were found. In women on combination drugs the HDL levels varied with the type and dose of the steroids but the general pattern was an increase of HDL cholesterol with increasing estrogen doses and a decrease with increasing dose or potency of the progestin component.

The clinical consequences of a reduced HDL cholesterol concentration in this group of young women remains to be elucidated. A low HDL concentration is a strong and independent risk factor for the development of atherosclerotic manifestations (14). No longitudinal studies have as yet shown whether lower HDL concentrations will be the cause of the increased risks to develop atherosclerotic manifestations that has been demonstrated in women on oral contraceptives (21). However as pointed out by Arntzenius *et al* in the Leiden study their 40 year old women on oral contraceptives had disturbingly low serum HDL cholesterol concentrations which were similar to the concentrations found in men of the same age group (2). A lowered HDL cholesterol for several years may place women at the same risk level as men for atherosclerotic manifestations.

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Submitted for publication January 5 1979

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# LOCAL APPLICATION OF KETOCAINE FOR TREATMENT OF REFERRED PAIN IN PRIMARY DYSMENORRHEA

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fect In a randomized double blind cross-over study effect on referred pain of the local anesthetic ketocaine evaluated in 23 women suffering from primary dysmenorrhea ketocaine was administered in compresses containing ketocaine in a 10 per cent ethanol solution The investigation comprised two consecutive menstrual periods During one the patient received ketocaine compresses and during the other placebo compresses i.e. compresses with ketocaine The compresses were applied to the skin area where the patient experienced the most intense pain When receiving ketocaine compresses 19 of the 23 patients reported good or moderate pain relief The effect was noticed within 30 to 60 minutes and lasted for a mean of 4.5 hours (range 1.5-5 h) Only four of the patients reported relief of pain when receiving placebo compresses This effect ceased when the compresses were removed No general side effects were registered However edema at the site of compress application was more often observed after ketocaine than after placebo compresses

menstrual pain is one of the most common gynecological complaints In the literature figures on prevalence of the disorder show wide variation (range 3-5-6-8) Preliminary results from a not yet completed study reveal that 15 per cent of school girls 15 years suffer from regularly recurring dysmenorrhea (10)

Dysmenorrheal pain may be the result of hyperactivity of the uterine muscle and myometrial ischemia Intravaginally performed intra uterine pressure recordings in healthy and dysmenorrheal women support this (1-11) The cause of the uterine hypercontractility is not known but it has been shown that  $\beta_2$  receptor stimulating agents (11) prostaglandin synthesis inhibitors (7) and recently calcium antagonists (1) can decrease uterine tone and intensity of uterine contractions in dysmenorrheal women Pain is associated with uterine relaxation

Many women suffering from primary dysmenorrhea localize their pain to the lower back or ab-

domen There is reason to believe that this pain is referred similar to that occurring in labor (2) In a previous investigation ketocaine a new local anesthetic drug was used to treat referred pain during the early stage of labor (9) The promising results of this study prompted us to investigate the effect of ketocaine in patients suffering from dysmenorrhea

## MATERIAL AND METHODS

Twenty three women with primary dysmenorrhea were studied All were well known to the investigators The patients suffered from moderate dysmenorrhea with the pain localized mainly in the back In 9 of the subjects intra uterine pressure recordings verified the presence of uterine hypercontractility during menstrual pain Vaginal examination laparoscopy hysteroscopy and/or hysterosalpingography excluded extrinsic factors as causes for the pain The investigation was performed as a randomized double blind cross-over study At the onset of the menstrual pain all 23 women received ketocaine compresses containing 1 g of active substance or compresses containing placebo i.e. without ketocaine A description of the compresses and the method of application has been given previously (9)

The investigation involved two consecutive menstrual periods

**Procedure** At the onset of menstrual pain the patient was admitted to the outpatient department of the clinic After observation for approximately one hour during which the need of pain relief was established compresses were applied to the skin area where the most intense pain was experienced Each compress was left in position for one hour During the time of compress application no analgesics were given

When the compresses were removed the patient's assessment of the effect of treatment was determined by using a simple scale 0=no effect X=moderate effect XX=good effect

Local effects on the skin such as erythema and edema were looked for and recorded The duration of the effect was estimated by asking the patient to record her degree of pain relief every second hour according to a written schedule She was also asked to record the amount of simple analgesics taken during the two following days

The same procedure was repeated at the next menstrual period

Table I Effect of treatment of assessed by the patients

	Good	Moderate	None
Placebo	1	3	19
Ketocaine	8	11	4

## RESULTS

As can be seen in Table I 19 of the 23 patients reported good or moderate pain relief after treatment with ketocaine compresses. The effect was recognized within 30 to 60 min and lasted in the mean for 3 h (range 1.5–5 h).

In contrast only 4 of the patients reported relief of pain when treated with placebo compresses. In three of the patients pain relief was reported within 30 min. The effect of treatment ceased when the compresses were removed.

Neither during treatment with ketocaine nor during the placebo administration were any general side effects observed. Local symptoms were reported both during placebo and ketocaine treatment (Table II). It was found that after ketocaine a few patients exhibited a moderate erythema at the site of compress application.

During the first two menstrual days the consumption of simple analgesics was 40 per cent higher in the group treated with placebo compresses compared with that treated with ketocaine.

## DISCUSSION

From the present study it is evident that ketocaine locally applied to the skin can be used to treat referred pain in primary dysmenorrhea. Similar results were obtained in patients suffering from painful labor (9). However, as dysmenorrheal pain lasts for a fairly long time and as the effect of the ketocaine compresses generally did not exceed three hours, reapplication has to be performed for effective pain relief during the day. Even though no marked local reactions on the skin were observed in the present study, reapplication of the ketocaine compresses may increase the risk of skin reactions (4).

Side effects of the commonly used drugs for treatment of menstrual pain — contraceptive pills, prostaglandin synthesis inhibitors and simple analgesics — are fairly common. The present results suggest that local treatment of referred pain with ketocaine might

Table II Percental distribution of reported symptoms at the site of compress application

Symptom	Placebo	Ketocaine
None	52	3
Cold	22	17
Warm	13	34
Burning	0	14
Itch	13	3

be an alternative in patients whose symptoms are moderate and last for a short time. Even if skin reactions or side effects can occur, the treatment is easily handled by the patient herself.

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Submitted for publication December 11, 1977

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## AMENORRHEA FOLLOWING ORAL CONTRACEPTION

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**Abstract.** In 21 patients with amenorrhea after taking oral contraceptives the urinary excretion of estrogens and the concentrations of LH, FSH and prolactin were studied. In five of these women the amenorrhea was accompanied by galactorrhea. Ten of the 21 patients had had amenorrhea before contraceptive therapy. The progestone test was negative in 9 patients and clomiphene test negative in 10 patients. The urinary excretion of estrogens and plasma LH and FSH values were low normal while prolactin concentration in group A (amenorrhea with galactorrhea) amounted to  $12.8 \pm 2.4 \mu\text{g/l}$  of plasma and group B (amenorrhea with galactorrhea) to  $85.4 \pm 15.8 \mu\text{g/l}$  ( $p < 0.01$ ). The pituitary response to 100  $\mu\text{g}$  synthetic HCG was similar to that found in the early follicular phase of the ovulatory cycle and showed that the pituitary is capable of synthesizing and secreting LH and FSH. In the three women from group A who wanted to become pregnant conceived under the therapy with Clomid (HCG) in all women from group B in response to clomiphene (bromocriptine) therapy administered in 5 mg dose the plasma concentration of prolactin decreased to a normal level, galactorrhea ceased within 15-62 days and resumed within 38-75 days. In three women it is probable that the cycle became biphasic and a previously clomiphene negative patient became a clomiphene responder.

Amenorrhea after treatment with combined oral contraceptives is encountered in clinical practice with increasing frequency. In many cases spontaneous restoration of the menses and of the ovulatory cycle occurs; however there are patients with persistent secondary amenorrhea who are often very resistant to therapy. Ten years after the introduction of oral contraceptives into clinical practice Whitlaw *et al.* (1966) reported on amenorrhea following cessation of oral contraception. Since then many papers have been published on the appearance of amenorrhea after using combined oral contraceptives (2, 6, 7, 8, 9, 10, 11) as well as on amenorrhea associated with galactorrhea (6, 10, 11). This paper presents the results of clinical and laboratory examinations in 21 patients with secondary amenorrhea which developed after discontinuation of treatment with combined oral contraceptives.

## MATERIAL AND METHODS

Twenty one patients with amenorrhea lasting for more than six months including five women with both amenorrhea and galactorrhea were studied. In all patients menarche had occurred at the normal age. Ten of our 21 patients had had oligomenorrhea before taking contraceptive pills. Fourteen of the women had already given birth and the remaining seven were nulligravidae. The adrenal and thyroid functions were normal in all women and plain roentgen rays of the sella turcica revealed no abnormalities. The ophthalmologic findings were also normal. The following determinations were carried out on all patients: the urinary excretion of total estrogens, the percentage of superficial cells in the vaginal smears (pycnotic index, PI), plasma LH and FSH concentrations by radioimmunoassay according to Midgley (12, 13) and of plasma prolactin by the method of Bryant *et al.* (4).

The LH-RH test was done by administering 100  $\mu\text{g}$  synthetic LH-RH in the plasma LH and FSH concentrations were measured before as well as at 15, 30, 45, 90, 120 and 240 min following injection. The progesterone test was performed by administering 4  $\times$  25 mg progesterone caproate in the morning and patients with a positive response were given 100 mg clomiphene daily between the 5th and 9th days of the cycle. The patients with a negative progesterone test were also given 100 mg clomiphene daily for five days beginning from the 10th day after the injection of progesterone. The clomiphene test was considered positive when the BBT was biphasic with a luteal phase longer than 12 days and the urine pregnandiol level higher than 2.5 mg/24 h between the 22nd and 24th day of cycle. The patients were divided in two groups. Group A consisted of 16 women with amenorrhea without galactorrhea and group B of 5 patients with both amenorrhea and galactorrhea. The basic clinical and laboratory findings of the patients are shown in Table I. Six of the 21 women had an infertility problem. Three patients from group A who wanted to become pregnant were given clomiphene and HCG. The 5 patients with amenorrhea and galactorrhea were treated with Parlodel (2-bromocryptine) 5 mg daily over a period of 2 to 5 months. The data obtained were statistically analyzed and the significance of the differences was tested using Student's *t* test at the 0.01 significance level.

## RESULTS

In all patients the basal estrogen concentrations and the pycnotic index were low and plasma LH and FSH concentrations were at the lower limit of normal.

Table I Clinical data of patients with amenorrhea after combined oral contraceptives

Group	Average age (years)	Average age at menarche (years)	Cycles before contraceptive treatment (normal/oligomenorrhea)	Duration of contraceptive treatment (months)	Duration of amenorrhea (months)	Progesterone test (pos/neg)	Cycles (pos/neg)
A (n=16)	26	14	9/7	20	11	11/5	12/4
B (n=5)	29	15	2/3	16	13	1/4	1/4

A = Amenorrhea without galactorrhea B = Amenorrhea with galactorrhea n = Number of patients normal/oligomenorrhea

for the early follicular phase. There were no significant differences between estrogen, LH and FSH concentrations as between the group with amenorrhea and the group with both amenorrhea and galactorrhea. In nine of 21 patients the progesterone test was negative. In group B the prolactin concentrations were significantly higher ( $p < 0.01$ ) than in group A (Table II). In one case galactorrhea appeared during the treatment with oral contraceptives and in the remaining 4 women after cessation of therapy. The results obtained by the LH-RH test are shown in Fig. 1. In both groups an increase in LH and a slightly smaller increase in FSH concentration was noted and the maximal concentrations were observed 60 min following the injection of LH-RH. The maximal increase in LH concentration was significantly higher in group A than in group B ( $p < 0.01$ ) while the maximal increase in FSH concentration was approximately the same in both groups. All five patients with amenorrhea and galactorrhea were given 5 mg of Parlodel daily and in the three women who wanted to become pregnant the plasma prolactin concentrations were determined weekly. All patients from this group measured their basal body temperature. In two patients from this group who did not want to conceive an IUD was inserted. Fourteen days after the in-

situation of Parlodel therapy prolactin concentrations were normal in all five women. Galactorrhea appeared within 15 to 62 days in four patients. In one patient a slight mammary secretion persisted beyond the 90th day in spite of the normal prolactin concentration. In all five patients spontaneous bleeding occurred within 38 to 75 days of therapy according to the BBT curve a biphasic cycle appeared in three patients while in two women the BBT showed a monophasic course even after 90 days of the establishment of monthly bleeding and cessation of galactorrhea. One of these women with a previously negative clomiphene test who wanted to become pregnant was given clomiphene in 100 mg daily doses from the fifth to the ninth days of the cycle and 10 000 IU of HCG on the fourteenth day. She did not become pregnant although ovulation was induced (Fig. 2).

Parlodel may change a clomiphene non-responder to a clomiphene responder. Two of the women from group A who were treated with HCG became pregnant but in one patient pregnancy could not be induced even with as high a dose of clomiphene as 150 mg daily over five days.

## DISCUSSION AND CONCLUSIONS

The role of oral contraceptives in the pathogenesis of secondary amenorrhea is not clear. Multiphase therapy may play a part in postpill amenorrhea and the irregularity of the menstrual cycle as a pre-factor has been reported by other authors (4, 5). Ten of the 21 patients with postpill amenorrhea and oligomenorrhea before the therapy. The progesterone tests obtained in nine of our 21 patients indicate severe estrogen deficiency. In 10 of these patients the results of the clomiphene test were negative or impaired (bleeding without ovulation). In a small group of patients with amenorrhea and galactorrhea both the progesterone and clomiphene

Table II Mean basic concentration of the hormones and pycnotic index in patients with amenorrhea after combined oral contraceptives

Group	TE µg/24 hr	PI	LH U/l	FSH U/l	Prolactin µg/l
A	18.0 ± 6.6	19.4 ± 7.4	12.0 ± 4.2	8.7 ± 2.5	12.8 ± 4.4
B	12.8 ± 4.7	15.8 ± 5.6	9.4 ± 2.8	7.3 ± 1.5	85.4 ± 15.8
	NS	NS	NS	NS	$p < 0.01$

TE = Total estrogens. Values are means ± standard error (normal values in follicular phase without midcycle peak: FSH 5–20 U/l, LH 5–15 U/l).

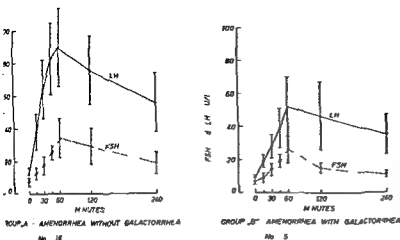
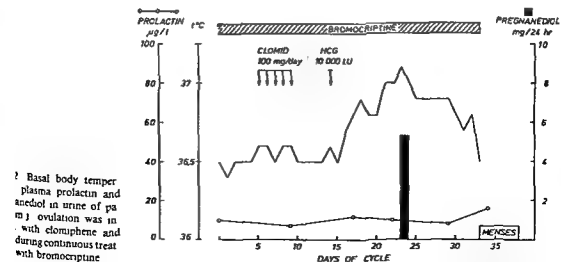


Fig 1 Plasma FSH and LH concentrations before and after administration of 100 µg LH RH : m (mean  $\pm$  SE)

negative in four of the five patients. Bohnet *et al* (3) observed a positive progesterone test in a high percentage of women with amenorrhea and an increased prolactin level and a negative clomiphene test in 15 of 17 patients. Franks *et al* (9) found that 100% of patients with amenorrhea and hyperprolactinemia had a negative progesterone test and 97% a negative or impaired clomiphene test. Stribanek *et al* (10) succeeded in inducing ovulation with clomiphene alone in only one of nine patients with amenorrhea and galactorrhea, while in the remaining eight it was necessary to give both clomiphene and human chorionic gonadotropin (HCG) and in some patients even human menopausal gonadotropin (HMG) and HCG. The pituitary capacity to synthesize and secrete gonadotropins was normal in both groups of patients. Basal LH and FSH

concentrations were low normal in both groups; the maximal increase in LH following LH-RH was significantly higher in group A than in group B ( $p < 0.01$ ) and the increase in FSH was approximately the same in both groups. Such a response was observed also in our previous investigation during the early follicular phase of the normal cycle (Smiljanic *et al* 1977, unpublished data). Similar responses have been found by Healy *et al* (11) while Dickey (6) has found a smaller LH increase following LH-RH in amenorrheic patients with increased prolactin and low basal estrogen and LH concentrations. In these women the response to clomiphene was negative. Floersheim, Shachar and Keller (8) have demonstrated that patients with the hyperprolactinemic anovulatory syndrome have an adequate pituitary response



to synthetic LH RH and a negative response to clomiphene. By the administration of Parlodel to patients with amenorrhea and galactorrhea it is possible to achieve suppression of the prolactin level restitution of menstruation and cessation of galactorrhea. Several other reports have been also published on the successful clinical use of Parlodel in patients with the anovulatory syndrome and hyperprolactinemia (6-8).

Parlodel as an agonist of dopamine stimulates the synthesis of the prolactin inhibiting factor (PIF) in the hypothalamus and acts upon the galactophores of the anterior pituitary by inhibiting prolactin synthesis and secretion (1).

It is a very suitable drug for the treatment of the anovulatory syndrome with hyperprolactinemia and its side effects are negligible.

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Submitted for publication January 1 1979

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## THE EFFECTS OF ESTROGENS AND GESTAGENS ON THE URETHRAL PRESSURE PROFILE IN URINARY CONTINENT AND STRESS INCONTINENT WOMEN

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**Abstract.** The present investigation was performed to study hydrodynamic effects of hormones on the lower urinary tract in women. Twenty-four stress incontinent and 6 continent women were randomly given  $E_2$  and  $E_3$  orally in doses of 8 mg per day for 3 weeks. Another group of 8 women given a single 1-m injection of 1000 mg gestagen. The women were examined with simultaneous urethrometry including urethral pressure profile measurements before and after treatment. After estrogen treatment transmission of intraabdominal pressure to the urethra ceased. Furthermore, there was a significant increase in minimum urethral pressure and urethral length at rest. After gestagen treatment no significant changes of the studied parameters were observed.

It has long been suggested that hormones, i.e. estrogen and progesterone, may affect the female urinary

tract. Most studies on this subject are based on the patients' subjective reports. However, objective studies, especially by intravesical and intra-urethral pressure recordings, have also been carried out. Thus Gitsch and Brandstetter (5) in 1954 registered an increased detrusor tone during the follicular phase and a decreased tone during the secretory phase of the menstrual cycle. In contrast, the urethral pressure increased during both the follicular and secretory phases. After treatment with estrogens, Slonsky (12, 13) registered a considerable improvement of female urinary incontinence. Walter *et al.* (14) found a subjective improvement of frequency, urgency and urge incontinence after estrogen therapy, but no significant changes in urethral pressure or urethral length after treatment. In a recently performed investigation, *et al.* (1979) did not obtain a positive effect on the parameters of importance for female stress incontinence after treatment with estrogens (4). The existing conflicting data concerning the value of estrogen treatment of female urinary incontinence is partly explained by different techniques of investigation and by different doses and types of estrogens used in the various studies.

The introduction of micro-transducer equipment has made it possible to study simultaneously and with high precision the bladder pressure, the urethral pressure and the urethral closure pressure both at rest and under stress situations (1, 2). With this technique, most of the measuring artifacts which afflict conventional pressure recording techniques have been eliminated, and reproducible recordings of high quality can be obtained. The present investigation was undertaken to evaluate the micro-transducer technique in the measurement of the effects of high doses of estradiol and estrin on parameters of importance for the maintenance of continence.

Further, the effects of progesterone on the lower female urinary tract seems to be uncertain, and few objective studies have been performed on this subject. The present study therefore also included an investigation of the effects of high doses of progesterone on the lower urinary tract.

## MATERIAL AND METHODS

Thirty-eight women took part in the study. Before the investigation they were informed of its purpose and gave their verbal consent. The patients were divided into two groups.

Group 1 (the estrogen group) consisted of twenty-seven postmenopausal and three premenopausal women with a mean age of 61 years (37-78), a mean body weight of 62 kg (49-75) and a mean parity of 1.7 (0-4). Twenty-four of the patients suffered from urinary stress incontinence (USI). The remaining six were continent. None of them had a cystocele, prolapse or urinary infection.

Table 1 Estrogen treatment

	MUP Cm H <sub>2</sub> O	CP Cm H <sub>2</sub> O	FUL mm	AUL mm
$E_2$ (16)	52/55	29/31	24/27	31/36
$E_3$ (11)	71/74	49/50	28/31	36/39
Total	59/63	37/39	25/28	33/37

mean values before and after treatment



Table II *Estrogen treatment Subjective and objective improvement of U S I*

Pat no	Age	Daily treatment	MUP Cm H <sub>2</sub> O	CP Cm H <sub>2</sub> O	PTR per cent	FUL mm	AUL mm
7	74	4 mg E <sub>2</sub>	36/37	16/9	66/148	25/24	31/3
9	70	4 mg E <sub>2</sub>	51/43	27/19	81/84	20/27	33/41
10	37	8 mg E <sub>3</sub>	111/116	91/91	?	37/31	47/5
11	66	8 mg E <sub>3</sub>	69/55	45/27	?	15/18	24/3
19	74	8 mg E <sub>3</sub>	65/65	41/37	37/87	34/28	40/38
21	60	4 mg E <sub>2</sub>	37/49	17/25	89/89	26/29	31/4
24	67	8 mg E <sub>3</sub>	47/48	19/24	72/83	25/26	40/42
30	72	4 mg E <sub>2</sub>	31/33	7/5	85/115	12/14	25/1
Mean	67		56/56	33/30		24/25	34/6

\*before and after treatment

Group II (the progesterone group) consisted of eight women all suffering from cystic glandular hyperplasia. Six were premenopausal and two postmenopausal. The mean age was 49 years (36–73), the mean body weight 58 kg (51–70) and the mean parity 1.6 (0–3). Five of the patients were continent and three stress incontinent. Relevant data of the patients are given in Tables II–VI.

The patients in Group I were randomly given estradiol valerate (Progynova<sup>®</sup> Schering A G) 4 mg daily for three weeks or estriol (Ovestrin<sup>®</sup> Organon) 8 mg daily for three weeks (Tables II–VI).

The patients in Group II received progesterone (17 hy droxyprogesterone caproate) (Primolut Depot<sup>®</sup> Schering A G) 1000 mg in a single intramuscular injection.

Immediately before treatment all patients were examined by simultaneous urethro-cystometry including measurements of the urethral pressure profile (UPP) according to a previously described technique (1, 2).

The bladder was filled with body warm saline at a rate of ml/min. To simulate a stress situation the patients were requested to cough after every 100 ml of saline had been infused. Calculations of the resting pressures were done at a bladder volume of 200 ml taken as a mean of three consecutive recordings.

Reinvestigation was weeks after the first examination.

Urinary incontinence was recognized in two ways:

- 1 By observation of leakage of urine from the urethral meatus
- 2 By observation of a negative urethral the recording curve (Fig. 1)

Definitions and abbreviations of *calculi*

Bladder pressure (BP) = maximum intravesical

Maximum urethral pressure (MUP)

Closure pressure (CP) = MUP minus BP

Pressure transmission ratio (PTR) = the percentage intra abdominal pressure transmitted to the relation to the bladder pressure on coughing

Functional length of the urethra (FUL) = that

urethra where the intra urethral pressure bladder pressure

Absolute length of the urethra (AUL) = that part

urethra where the intra urethral pressure

atmospheric pressure

Pressure in cm H<sub>2</sub>O Length in mm

Statistics: The differences observed during the study

tested for significance by the Student's *t* test

Table III *Estrogen treatment Subjective but no objective improvement of U S I*

Pat no	Age	Daily treatment	MUP Cm H <sub>2</sub> O	CP Cm H <sub>2</sub> O	PTR per cent	FUL mm	AUL mm
5	62	4 mg E <sub>2</sub>	61/65	37/33	72/76	16/20	23/29
12	60	4 mg E <sub>2</sub>	64/67	44/47	56/88	26/26	33/38
13	52	8 mg E <sub>3</sub>	73/76	58/52	?	26/26	37/45
16	49	8 mg E <sub>3</sub>	89/91	65/67	?	35/38	45/4
17	69	4 mg E <sub>2</sub>	49/61	25/33	88/70	25/41	31/4
20	51	4 mg E <sub>2</sub>	65/59	45/39	80/70	24/25	28/3
22	52	4 mg E <sub>2</sub>	56/61	40/41	?	24/23	41/4
23	57	8 mg E <sub>3</sub>	59/56	39/36	72/73	29/44	27/4
27	54	8 mg E <sub>3</sub>	63/77	47/36	?	25/37	31/4
Mean	57		64/68	44/42		26/31	

before and after treatment

Table IV Estrogen treatment No subjective no objective improvement of U S I

no	Age	Daily treatment	MUP Cm H <sub>2</sub> O	CP Cm H <sub>2</sub> O	PTR per cent	FUL mm	AUL after mm
66		4 mg E <sub>1</sub>	41/39	17/23	86/63	16/18	24/29
50		4 mg E <sub>1</sub>	43/45	21/29	81/90	21/20	25/27
III		4 mg E <sub>2</sub>	67/60	37/40	?	27/27	35/37
64		4 mg E <sub>2</sub>	45/52	17/24	?	19/20	26/25
53		4 mg E <sub>2</sub>	36/47	12/22	69/66	26/23	33/30
45		8 mg E <sub>3</sub>	99/120	79/96	?	30/39	33/41
50		4 mg E <sub>2</sub>	61/59	37/39	?	19/25	28/30
I	56		56/63	32/39		23/24	29/31

before and after treatment

## RESULTS

## Group I

**Urethral pressure** The MUP at rest increased in Group I after estrogen therapy from a mean of 59 cm H<sub>2</sub>O to a mean of 67 cm H<sub>2</sub>O ( $p < 0.05$ ) (Table I). There was no difference between continent and stress incontinent patients. Moreover, no difference in effect was found between estrinol and estradiol treated patients (Tables I–V).

**Bladder pressure** A non significant increase in BP from a mean of 24 cm H<sub>2</sub>O to a mean of 24 cm H<sub>2</sub>O was observed after estrogen treatment. No difference was observed between the estrinol and estradiol treated patients in this respect, and no difference between stress incontinent and continent patients.

**Urethral closure pressure** In accordance with the changes in the urethral and bladder pressures, urethral closure pressure did not change significantly after the estrogen therapy: 37 cm H<sub>2</sub>O before and 39 cm H<sub>2</sub>O after treatment, respectively.

**Functional urethral length** The FUL increased significantly in patients in Group I after estrogen therapy from a mean of 25 mm to a mean of 28 mm ( $p < 0.05$ ). There was no difference between estrinol

and estradiol treated patients and no difference between continent and incontinent patients.

**Absolute urethral length** The AUL increased from a mean of 33 mm to a mean of 37 mm after estrogen treatment (Table I). This change is significant ( $p < 0.05$ ). In common with the findings of FUL there was no difference between estrinol and estradiol treatments and no difference between continent and incontinent patients, respectively.

**Subjective versus objective effects on U S I** Seventeen of 24 patients suffering from stress incontinence reported subjective improvement of their symptoms. However, there was no correlation between subjective improvement and the improvement of MUP, CP or the urethral lengths (Tables II–V).

**Intra-abdominal pressure transmission ratio to the urethra** In eighteen of the twenty seven patients of Group I the PTR was determined during coughing. In six patients the pressure transmission was considerably improved after estrogen therapy (Tables II–V). This improved pressure transmission was highly related to the patients' subjective report of improvement. Thus, all seven patients with improved pressure transmission reported subjective improvement after

Table V Estrogen treatment Continent women

no	Age	Daily treatment	MUP Cm H <sub>2</sub> O	CP Cm H <sub>2</sub> O	PTR per cent	FUL mm	AUL mm
54		8 mg E <sub>3</sub>	59/67	39/47	?	33/34	36/37
58		4 mg E <sub>2</sub>	48/59	28/39	105/82	37/46	41/60
III		8 mg E <sub>3</sub>	63/64	43/44	88/83	22/25	29/28
76		4 mg E <sub>2</sub>	64/61	36/33	75/92	33/47	45/58
65		8 mg E <sub>1</sub>	56/56	24/32	?	26/23	41/32
66		4 mg E <sub>1</sub>	77/85	61/65	62/89	28/29	31/36
III	63		61/65	38/43		30/34	37/42

before and after treatment

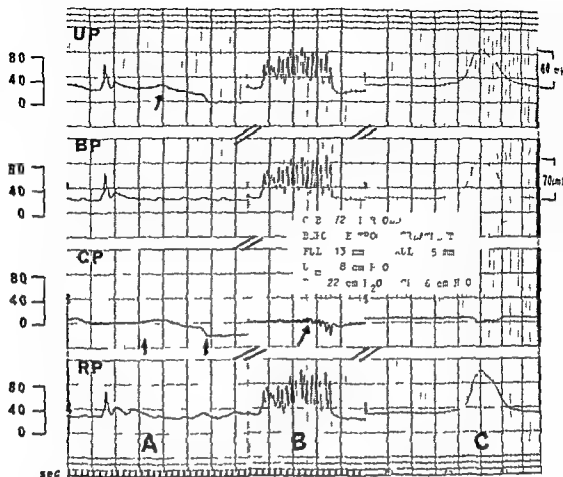


Fig 1 In section A the urethral pressure profile (arrow) in patient G II before estrogen treatment is shown. In section B a negative cough profile (arrow) is recorded i.e. the CP is negative throughout the urethra indicating stress in continence. In section C the urethral transducer is located at the urethral peak pressure. A cough resulting in a pressure increase of 70 cm H<sub>2</sub>O is transmitted to the bladder and 60 cm H<sub>2</sub>O to the urethra i.e. a urethral pressure

transmission (PTR) of 85 per cent. The cough results in a negative CP.

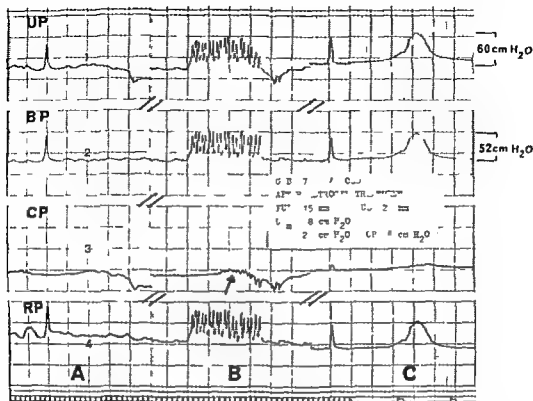
Catheter withdrawal speed in sections A and B 2 mm/sec. Paper speed 2.5 mm/sec. In section C paper speed 1 mm/sec.

UP = Urethral Pressure BP = Bladder Pressure CP = Cough Pressure RP = Rectal Pressure FUL = Functional Urethral Length AUL = Absolute Urethral Length.

Table VI Gestagen treatment

Patient no	Age	MUP Cm H <sub>2</sub> O	CP Cm H <sub>2</sub> O	PTR per cent	FUL mm	AUL mm
A	52	93/76	65/52	?	30/22	45/38
B	48	79/83	43/59	58/52	25/26	40/44
C	48	129/112	109/88	?	37/38	45/43
D	42	81/89	61/69	81/54	26/26	31/30
E	73	80/73	52/49	?	24/27	31/41
F	41	108/108	84/88	107/76	40/40	50/30
G	49	72/84	52/64	?	29/26	35/31
H	36	68/93	60/73	?	34/31	35/40
Mean	49	89/90	66/68		31/30	39/40

before and after treatment



Same patients as in Fig 1 after estrogen treatment. Type of recording in the different sections. Note that is unchanged compared with Fig 1. In spite of this a

positive cough profile is shown (arrow section B). The intraabdominal pressure transmission to the urethra has increased to 115 per cent.

ment (Tables I–II) and five were shown to be actively continent. Figs 1 and 2 exemplify the findings from one of these patients.

#### Group II (Gestagen treated subjects)

As can be seen from Table VI none of the recorded parameters changed significantly at rest after gestagen treatment. In three patients where it was possible to determine PTR, this parameter showed lower values after gestagen treatment in all three patients.

### DISCUSSION

The present investigation has clearly shown that even despite high doses over a long period, inducing only small changes in important continence parameters. Although an increase in urethral pressure length was obtained after the treatment, it is hard to believe that these small changes alone might imply a definitive improvement of urinary incontinence.

This suggestion is supported by the observation that there was no significant relation between subjective improvement of the symptoms of urinary stress incontinence and the increase of UP and urethral lengths (Tables II–III). The findings in this respect agree with those reported by Walter *et al.* (14) and by Ek *et al.* (4) but disagree with the results reported by Raz *et al.* (9) and by Schreiter *et al.* (11).

These different results can be explained by the different techniques of investigation used. The present study was performed with the micro-transducer technique, whereas the investigations performed by Raz *et al.* and by Schreiter *et al.* were carried out with the aid of conventional open-end catheters with constant flow.

One explanation for the often reported subjective improvement of urinary incontinence after estrogen therapy in postmenopausal women could be that before treatment leakage of urine causes a burning sensation on the atrophic vaginal mucosa. Since estrogen

treatment causes a trophic mucosa urinary leakage may be recognized after treatment as less painful or even disregarded by the patients

An important observation in the present study was that in 7 of our patients with subjective improvement of stress incontinence after treatment with estrogen an improved transmission of the intraabdominal pressure (PTR) to the urethra was observed (Tables II–III Figs 1–2). A definite explanation for this cannot be given but it may be due to factors outside the urethra e.g. improved function of the pelvic floor muscles and the para urethral connective tissues. It is interesting to note that in a recently performed urethro cystometric investigation of young continent women before and after treatment with danazole it was found that despite considerable decreased plasma estrogens the urethral pressure profile at rest was almost unaffected whereas the PTR decreased in several of the patients (8).

The findings that gestagens did not affect the present recorded parameters are at variance with the results reported by Raz *et al* (9) but agree with those obtained by Rud (10). In an investigation of young nulliparae during the menstrual cycle this author could not find any significant changes in urethral pressure bladder pressure or urethral lengths related to the changes of progesterone in the plasma.

It may be concluded that estrogens in high doses over a long period can improve female stress incontinence mainly by an improved pressure transmission of intra abdominal pressure to the urethra. It must however be emphasized that estrogens in doses used in the present investigation may induce adverse effects on other organs e.g. thromboembolic diseases (3–6) or uterine carcinomas (7). It is therefore debatable whether such therapy is justified in the treatment of patients with symptoms of urinary incontinence alone.

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Submitted for publication September 6 1979

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# THE IMPACT OF CYTOLOGICAL SCREENING ON THE INCIDENCE OF INVASIVE CERVICAL CANCER

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A cytological screening program for the early detection and prevention of cervical cancer was started in the county of Malmöhus, southern Sweden in 1967.

The incidence of invasive cervical cancer among women regularly attending the screening program has been reduced by 10 years to about one quarter of the incidence before the initiation of the program.

Results calculated from the whole female population did not indicate a significant decrease in incidence. Adequate individual follow-up combined with standardized administrative programs and population registers seem to be necessary whenever the effects of a mass screening program are evaluated.

Previous reports from British Columbia and other countries give substantial support to arguments for the value of cytological mass screening. Statistical analysis also indicates that the decrease in incidence of cervical cancer is much slower than was expected in Sweden, each citizen has an individual identification number which is in everyday use.

In 1967 a cytological screening program was started in southern Sweden in the county of Malmöhus (M). Cytological results as well as some other clinical data have previously been reported (13). In the present paper the impact of this program on the incidence of invasive cervical cancer in the M county is reported. In addition, the incidence of cervical cancer in two other counties — Kronoberg (G) and Jönköping (F) is reported. The screening program in these counties was started in 1970.

## MATERIAL AND METHODS

The total number of women in M county in 1965 was 250 000 and by 1975 the number had risen to 250 600. All women between 30 and 49 years of age (57 000) were invited to participate (13) and a quarter was invited every year. The first follow-up runs through 1973 and thus most women have been invited twice. The female population has been divided into three categories: those who attended the program

(68 per cent), those considering themselves under regular control (18 per cent) and those refusing or ignoring the offer (9 and 5 per cent respectively). In tables and diagrams of the programs from counties F and G only those women who actually participated in the screening program are included.

In general, the screening was completed during the first year. During the following years all non-negative findings were reevaluated and complementary diagnostic procedures as well as treatment were started.

From 1967 to 1973 all patients with invasive cervical cancer in the above-mentioned areas were investigated with particular emphasis on how the diagnosis of invasive cancer was obtained in the previous cytological examinations. When appropriate statistical background data from the population registry and data derived from the computerized screening programs are given.

The incidence is given as the average of the four separate 5-year cohorts included in the program. The year when the first screening procedure was performed is indicated as year number 0.

## RESULTS

In Fig. 1 the crude frequency of invasive cervical cancer in the county of Malmöhus is given. Around 1958–59 cytological examination became widely adopted as a diagnostic method in M county. The corresponding peak in the frequency curve is likely to be a reflection of an initial increase in the detection

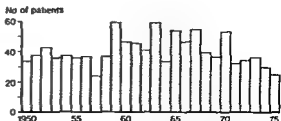


Fig. 1 Crude frequency of invasive cervical cancer in county of Malmöhus (M county) since 1950. Cytologic cervical screening was generally adopted around 1958–1960.

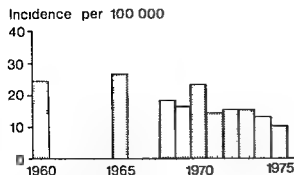


Fig 2 Incidence per 100 000 women of invasive cervical cancer in the whole population of M county

rate. However the initiation of the cytological screening program in 1967 was barely detectable and a statistical analysis of the crude data did not demonstrate a significant impact on the frequency of invasive cervical cancer nor could a significant decrease in the incidence in the whole population be demonstrated (Fig 2).

There was however a significant lower incidence in the age groups participating in the screening program as well as among the women considering themselves under regular control (Fig 3).

The incidence of invasive cervical cancer among the abstaining women remained the same before and after the screening.

In Fig 4 the incidence figures for the group women attending the screening program are presented. Before the year of re screening the curve represents the incidence of invasive cervical cancer in the corresponding year cohorts.

The incidence of invasive cervical cancer in the counties M, F and G demonstrated a marked decrease a few years after the first screening.

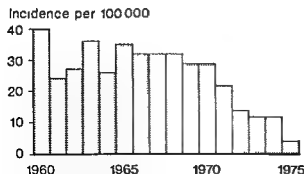


Fig 3 Incidence per 100 000 women of invasive cervical cancer in age group 30-49 years in M county

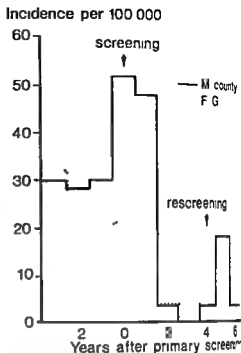


Fig 4 Incidence per 100 000 women of invasive cervical cancer among women properly attending the screening program in M county and in the counties F and G

## DISCUSSION

The ultimate goal of the screening program is a decrease in the mortality rate of cervical cancer. This has been recorded in only few reports (5, 19). The unreliability of mortality statistics in most countries obviates any far reaching conclusions on the impact of the screening programs. A more useful evaluation of the impact of cytological mass screening programs is the number of women with a diagnosis of invasive cervical cancer. Assuming that the therapeutic results, the stage distribution, the biological properties of the malignant disease are unaltered, a decrease in the incidence of the disease should indicate a diminished mortality.

The crude frequency of invasive cervical cancer in M county has not significantly altered during the 25 years. The incidence curve did not demonstrate a significantly decreasing slope (Fig 2) which is in accordance with other observations (7, 9, 12, 14).

However, if the incidence is calculated in the age group of the population that since 1967 has been included in the screening program in the county, a marked decrease in incidence is recorded (Fig 3). This is in accordance with other reports (2, 3, 4, 8, 15). An even more apparent reduction in the incidence of the screening program if incidence figures

lated from that part of the population that actually participated in the program (Fig. 4). The reduced incidence could first be noted 3 years after the start of the screening program. The clear increase was found during the first two years, is most likely a reflection of the increased diagnostic activity. The increase in the incidence of rescreeing observed after 3 years is likely to be explained by the number of women who entered the screening program for the first time at the rescreeing (approximately 30 per cent). Of these the majority moved into the county during the interval between the first screening and rescreeing. Some women in this group did not respond to the first invitation but joined the program at a second opportunity.

The pronounced and rapid decrease in the incidence of invasive cervical cancer has not been observed by other epidemiologists. The difference could be explained by the fact that when incidence calculations are calculated from the whole population the effect cannot be detected before several years have elapsed, whereas when the calculations are made from incidence figures in that part of the population which actually was screened the effect is noted early. In most western countries the proportion of older women in the whole population is steadily increasing. This might contribute further to the weak impact of the screening program on the total number of invasive cervical cancers in the female population as a whole. We suggest that a reduced incidence might be explained by fluctuations or a steady decrease caused by unknown factors influencing the natural history of cervical cancer (1, 6).

The screening program started 3 years later in the counties F and G and was confined to the same age group. In these counties identical results were observed which might indicate that the observed reduction in the incidence of invasive cervical cancer is a result of the screening program.

## CONCLUSION

The reduced incidence of invasive cervical cancer was observed a few years after our cervical mass screening program was started. However, the impact was detected only in the screened part of the population and not in the total population, even 10 years after the screening program was started. Adequate population statistics combined with a thorough individual follow-up seem to be mandatory for evaluating the effect of mass screening programs.

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Submitted for publication February 28 1979

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## ANNOUNCEMENT

**The 1st American Congress of Andrology** will take place in Mexico City Americana Fiesta Palace Hotel January 26–31 1981

*Information requests for registration and travel information should be directed to*

Dr A Negro Vilar  
University of Texas Health Science  
Center at Dallas  
Southwestern Medical School  
Department of Physiology  
5323 Harry Hines Boulevard  
Dallas Texas 75235  
USA

*Information requests for registration and travel information should be directed to*

Gerald Bagatzinski  
Congress Administrator  
31600 West Chicago  
Livonia Michigan 48150  
USA

**The 11th World Congress of Human Reproduction** is to be held in West Berlin March 22–26 1981

*Main topics*

- Central nervous system and regulation of reproduction
- Surgical and morphological aspects of reproduction in men and women
- Gonadotrophins and their target tissue  
Beginning of life *in vivo* and *in vitro*  
Experimental embryology of mammals  
High risk reproductive factors in breast and genital cancer
- Influence of the environment and drugs on reproduction  
Free Communications  
Film Festival

*Information*

Congress Secretariat  
Priv Doz Dr L Mettler  
Frauenklinik der Universität  
Hegewischstrasse 4  
D 2300 Kiel 1  
West Germany

**The Third International Congress on the Menopause** held in Belgium at the coastal resort of Ostend on 9–12 1981 just before the holiday season begins

This Congress will differ from the previous two (Grande Motte 1976 and in Jerusalem 1978) in that as the aim in the past was to arrive at a consensus of opinion this time we shall bravely attempt to confront the controversies. It will however take the same form as the ones — a number of small workshops rather than a meeting attended by everyone.

*For further information*

The International Menopause Society  
8 av Don Bosco  
B-1140 Brussels  
Belgium  
Tel (02) 771 95 00 and (02) 771 96 45

**The Eighth Asian Congress of Obstetrics and Gynaecology** will be held in Melbourne in October 1981

An exciting and varied programme will be presented including expert plenary speakers from Asia Australia and other Centres. The Congress themes include

- Population Control
- Maternal and Perinatal Mortality
- Trophoblastic Disease  
Gynaecological Malignancy  
Obstetrical and Gynaecological Endocrinology

The official language will be English  
*Further information may be received from*

The Organizing Secretary  
VIIIth Asian Congress of  
Obstetrics and Gynaecology  
G P O Box 2195T  
Melbourne 3001  
Victoria  
Australia

## CASE REPORT

## AMNIOCENTESIS IN TWIN PREGNANCIES UNDER GUIDANCE OF THE GLUCOSE CONCENTRATION IN AMNIOTIC FLUID

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Prenatal diagnosis of twin pregnancies is necessary to mark the cavity punctured first to ensure aspiration of amniotic fluid from both amniotic sacs. Congo red (1) and methylene blue (3-6) have only been used for this purpose. It has been difficult to obtain the right form of congo red in Denmark and methylene blue might be contra-indicated in serious complications have been described in its receiving this preparation (4, 10). This difficulty led us to the idea that a biological substance already present in the amniotic fluid might be a better marker. Glucose is present in the amniotic fluid in the second trimester in a normal concentration of about 0.3-0.6 g per l (8) and even in higher concentrations it is supposed not to harm the fetus. The use of glucose as a marker requires a quick test for its determination. The Dextrostix system (Ames) fulfils this requirement.

## MATERIAL AND METHODS

The principle of the Dextrostix Eytone system is an electrical evaluation of the color shift on a strip which has been immersed in the glucose solution. The system has been used for determination of the glucose concentration in whole blood (9) and in serum (7) by reduction of the reaction time to 45 seconds. For determination in amniotic fluid a special device was placed in a small test tube and the Dextrostix is immersed for 45 seconds. With this exception the directions of use of the apparatus have been followed strictly. The amount of amniotic fluid at the 15-16th week of gestation is 125-175 ml (5). At this time the injection of 2 ml isotonic glucose into the amniotic cavity will increase the glucose concentration 2-3 times. By establishing this method we had to be sure that the augmentation of the glucose concentration takes place quickly and that it remains high during the period of the investigation. In a patient who had a provoked abortion we found that the concentration reached a high and constant level within two minutes and remained unchanged for more than half an

hour. The ultrasonic scanning was performed with the Ecoline ultrasonic apparatus. After localization of the placenta the optimal sites of insertion in the uterus were determined by consideration of the fetal echoes. The first needle insertion, the more difficult one to perform, was carried out using a puncture transducer (2) while the second insertion was performed free hand. Fifteen milliliters of amniotic fluid were aspirated by the first puncture and 1 ml of this was used for determination of the glucose concentration. Through the puncture needle a suitable amount of isotonic glucose solution was injected, good mixing being ensured by aspirating and reinjecting before the glucose concentration was reestimated. From the second site of insertion 1 ml of amniotic fluid was aspirated for determination of the glucose concentration and if this was in accordance with the physiological level we aspirated 15 ml of amniotic fluid through this puncture needle for prenatal diagnosis. Finally it was controlled that the glucose concentration in the first cavity was still much higher than in the second cavity punctured.

**Case 1** is a primigravida aged 39 years. The indication for the amniocentesis in her 16th week of gestation was aetasia. The placenta was localized to the posterior wall and the right upper part of the anterior wall. The first puncture was performed close to a caput at the upper and left part of the anterior wall of the uterus. Clear amniotic fluid was aspirated with a glucose concentration of 0.7 g per l. After injection of 2 ml isotonic glucose solution the concentration increased to 1.3 g per l. The second puncture was performed close to a caput in the lower and right part of the anterior wall of the uterus and clear amniotic fluid with a glucose concentration of 0.8 g per l was removed. Finally the glucose concentration in the cavity punctured first was controlled and found to be 1.4 g per l. Cytogenetic examination of cells from the amniotic cavities showed the normal male karyotypes 46 XY with identical chromosome markers in preparations stained with Quinacrine Mustard and Giemsa for C banding, indicating that the fetuses were monozygotic twins. The patient gave birth to living male twins in the 39th week of gestation. One placenta with a double bladdered septum was found. Cytogenetic examination of leucocytes from peripheral blood from the children using the same method as described above showed identical chromosome markers which confirmed that the twins were monozygotic.

**Case 2** is a 4th gravida aged 41 years. The indication for amniocentesis in the 15th week of gestation was aetasia. The

placenta was localized to the lower part of the anterior wall of the uterus and two heads could be demonstrated in the upper part of the amniotic cavity. The amniocentesis was performed in a similar manner to that described above. The glucose concentration in the cavity punctured first was 0.4 g per l before and 1.1 g per l after injection of 2.5 ml isotonic glucose solution into the cavity. The concentration in the other cavity was 0.1 g per l and the control concentration from the first cavity was 1.85 g per l. Cytogenetic examination of cells from one cavity showed a normal male karyotype 46 XY and from the other cavity a normal female karyotype 46 XX. A comparison of the heteromorphic fluorescent markers in the chromosomes from the female fetus with the chromosomes from the mother in order to exclude the possibility of contamination with maternal cells showed clear differences in chromosome pairs Nos. 13, 14, 15 and 21. The patient has not yet been delivered.

### CONCLUSIONS

We conclude that the Dextrostix Eyetone system for the determination of glucose concentration is sufficiently fast and accurate for the present purpose. The glucose concentration in amniotic fluid after the injection of isotonic glucose remains at a high level long enough for the amniocentesis to be performed. These circumstances make the method useful in practice. The use of glucose as a marker in amniocentesis in cases of twin pregnancies seems to be without any risk since we are dealing with a biological substance which is already present in the amniotic fluid and which takes part in the normal metabolism of the fetus. Therefore glucose is preferable as a marker to the above mentioned dyes.

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Submitted for publication March 1, 1979

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## CASE REPORT

## POSTCESAREAN VESICO UTERINE FISTULA

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A case of postcesarean vesico-uterine fistula in connection with a fibrous cord joining the anterior and posterior wall of the bladder is reported. The symptoms of hematuria and amenorrhoea in the absence of urinary incontinence are explained by reference to the pressure gradients which normally exist in the bladder and the uterine cavity. Urology, diagnosis and therapy of this and other rare cases are reviewed.

Uterine necrosis following prolonged labor used to be the dominant cause of obstetric urinary fistulae nowadays reported only from developing countries. Such fistulae are usually formed between the bladder and the vagina. In a report by Bird (2) of 70 urinary fistulae 65 were seen after prolonged labor. Thirty seven were classified as vesico vaginal or as vesico-urethral vaginal and one as a urethral fistula. The remaining 5 were between the bladder and the uterus. 2 of them were of the uterine type and developed after lower segment cesarean section and 3 were of the vesico cervical type and were secondary to spontaneous rupture of the lower uterine segment and the bladder. The bladder was repaired at the same time as the ruptured uterus but the repair was unsuccessful resulting in fistula formation. Bird (8) described four different types of fistulae: 1) to cesarean section, the vesico vaginal fistula, the vesico cervico-vaginal fistula, the vesico-cervical and the vesico uterine fistula. The vesico cervical and the vesico uterine fistulae are usually connected with amenorrhoea and cyclic hematuria (4, 6). Urinary fistulae are however not associated with urinary incontinence in spite of a patent cervical fistula. Cyclic hematuria has been named menohematuria by Youssef (9). The symptoms are more or less dependent on how long the patient lactates. Vesico-uterine fistulae are rare and as a rule caused by an expertly performed cesarean section where

the bladder has not been properly mobilized and sometimes inadvertently entered. Consequently the risk of developing a fistula of this type is increased in patients where previous operations have resulted in a fixation of the bladder to the anterior uterine wall.

We report a case of vesico uterine fistula where pre operative investigation also revealed a fibrous bridge between the anterior and posterior wall of the bladder associated with other urologic symptoms.

## CASE REPORT

A 32 year-old woman who 4 years previously had undergone surgery because of thyrotoxicosis was admitted 4 months after cesarean section complaining of hematuria, frequency and dysuria. The patient's first pregnancy resulting in a live male infant was terminated by a transverse lower segment cesarean section because of disproportion between the size of the pelvis and the head of the fetus. At operation the bladder was accidentally opened. The opening measured 4 to 5 cm and was after delivery closed with two rows of isolated catgut. A Foley catheter was used to drain the bladder for 2 weeks. In the immediate post-operative period hematuria and albuminuria were seen. The patient was discharged after 3 weeks.

However frequency and dysuria continued and 2 months later massive hematuria occurred. The hematuria gradually disappeared and the urine cleared. There was no vaginal discharge and no menstrual blood was noted at the same time as hematuria. The patient's complaints resulted in further investigation before admission. An intravenous pyelogram (IVP) was normal. A cystogram revealed an irregular bladder and a diverticulum was described anteriorly to the right. When hematuria was again noted a month later the patient was referred to our department.

It was now clearly established that no hematuria had been seen before lactation ceased. At the time of her periods which she could feel massive hematuria occurred for a couple of days and then gradually disappeared over the next few days. She did not notice any vaginal bleeding.

On admission physical examination revealed a nicely healed umbilical incision. The vagina was normal. The uterus was retroflected and of normal size. Speculum examination showed a small central erythroplakia on the cervix.

placenta was localized to the lower part of the anterior wall of the uterus and two heads could be demonstrated in the upper part of the amniotic cavity. The amniocentesis was performed in a similar manner to that described above. The glucose concentration in the cavity punctured first was 0.4 g per l before and 1.85 g per l after injection of 2.5 ml isotonic glucose solution into the cavity. The concentration in the other cavity was 0.48 g per l and the control concentration from the first cavity was 1.85 g per l. Cytogenetic examination of cells from one cavity showed a normal male karyotype 46 XY and from the other cavity a normal female karyotype 46 XX. A comparison of the heteromorphic fluorescent markers in the chromosomes from the female fetus with the chromosomes from the mother in order to exclude the possibility of contamination with maternal cells showed clear differences in chromosome pairs Nos. 13, 14, 15 and 21. The patient has not yet been delivered.

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Submitted for publication March 1, 1979

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Cystogram during micturition. Oblique projection. Fistulous tract joining the bladder and the uterine cavity marked with an arrow.

The absence of urinary incontinence is interesting. In this patient there was no apparent cervical stricture and she menstruated vaginally after the fistula had been closed. Youssef (9) suggested a functional valve in the cervical portion of the fistula but was unable to localize such a valve. Instead he proposed that

there might be sufficient pressure in the isthmus sphincter leading the menstrual blood into the bladder. This latter explanation was however regarded as unlikely by Tancer (8) who proved that amenorrhea and cyclic hematuria persisted without urinary incontinence even if the uterine fistulous tract was localized below the anatomical functional isthmus.

We believe that the explanation might be found in the pressure gradients seen between these two hollow organs. Normally the pressure in the filling phase of the bladder in females rarely exceeds 20 cm H<sub>2</sub>O (10). When the detrusor muscle contracts the intravesical pressure in females rises to but rarely exceeds 40 cm H<sub>2</sub>O. Normally the intravesical pressure during voiding in females is between 20 and 40 cm H<sub>2</sub>O. Intra-uterine pressure varies and depends on the phase in the menstrual cycle. It is highest during menstruation when pressures between 130 and 160 cm H<sub>2</sub>O have been recorded. In the intermenstrual phase pressures are substantially lower. In the proliferative phase pressures between 35 and 70 cm H<sub>2</sub>O have been recorded and in the secretory phase pressures are higher 55 to 100 cm H<sub>2</sub>O (11). Therefore the pressure in the uterine cavity seems to be higher than the pressure in the bladder except during micturition.

When a patient with a vesico-uterine fistula voids there may be a flow of urine from the cervix because of the increased intravesical pressure but this may be difficult for the patient to observe.

In this case it was possible to localize the fistulous tract by means of a cystogram as in the case reported by Sammour (7). In his case hystero-salpingography



Photo at operation after opening the bladder. The fistulous tract joining the anterior wall is seen (arrow). A cannula is introduced into the fistulous opening.

did not show any communication between the uterus and the bladder. We did not try to visualize the fistula by hystero salpingography which was done in the case reported by de Carvalho (3). The possibility of visualizing the fistula seems to depend on the pressure applied during the cystogram and/or the hystero salpingography and the phase in the menstrual cycle.

The diagnosis is easily established by the history if the examining doctor is familiar with this syndrome. The combination of cesarean section, amenorrhea and cyclic hematuria in the absence of urinary incontinence is pathognomonic for a vesico uterine fistula. In order to further delineate the exact localization of the fistula, urethro cystoscopy and roentgen ray examinations such as cystography and/or hystero salpingography may give further information.

In our case there was also a dense fibrous bridge between the anterior and the posterior wall of the bladder. Residual urine amounted to 24 ml and the patient had a urinary tract infection. The symptoms of frequency and dysuria can be explained by the infection and the fibrous bridge in the bladder. These symptoms disappeared after division of the bridge and treatment of the urinary tract infection. The etiology of the bladder lesion was a cesarean section but in this case the bladder was closed in a way that joined the anterior and posterior wall.

The management of this type of fistula is very much the same as for other types of urinary fistulae. Transabdominal repair, opening of the bladder and localization of the opening of the fistula is recommended for those not familiar with vaginal surgery. The dissection of the fistulous tract between the ventral surface of the uterus and the bladder is facilitated and more safely performed if ureteral catheters are inserted. The uterine and vesical portions of the fis-

tula should be excised and closed and it is advised to interpose a pedicled omental flap and secure it to the suture line.

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Submitted for publication March 14, 1979

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## CASE REPORT

## INCORRECT DIAGNOSIS OF PLACENTAL INSUFFICIENCY IN A PATIENT WITH PRE ECLAMPSIA

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A case of pre eclampsia is described where suspicion of placental insufficiency arose due to low estradiol levels and a positive oxytocin challenge test (OCT). The cause of these findings was later proven to be strictly fetal. It is concluded that low estradiol levels and a positive OCT are caused by a decreased placental capacity but specific of placental function as for instance estimations of estradiol in plasma and/or a DHAS-test should be used to verify or rule out a suspicion of placental insufficiency.

As in cases of placental sulphatase deficiency (7) the capacity of the placenta to form hormones seems to parallel the placenta's nutritive and respiratory function. This is the basis for using hormone assays in the management of high risk pregnancies. Some of the hormones such as HPL are entirely placental in origin whereas some steroids in particular estradiol are formed in the placenta from fetal precursors (2). Antepartum monitoring of the fetal heart rate pattern in response to uterine contractions (OCT) is a

widely used test for evaluation of placental function (4). A fetal heart rate pattern with late decelerations is believed to indicate uteroplacental insufficiency (4).

## CASE REPORT

A 32 year old woman gravida IV para II was admitted to the hospital in the 37th week of pregnancy due to hypertension and headache. The gravidogram (8) was without obvious deviations. An ultrasonic measurement of the fetal biparietal diameter was done and found normal (92 mm). Her urinary excretion of estradiol however was below normal (6) on 3 consecutive days. An antepartum OCT (Fig 1) showed clearly defined late decelerations. With these facts at hand it was presumed that this pregnancy was complicated by a placental insufficiency and the pregnancy was terminated by a cesarean section. The child, a boy weighing 3010 g, had a severe malformation of the heart. He died at an age of 5 days.

A DHAS-test (1) was performed the day before delivery. The result which was not available when the decision of premature intervention was made showed that the placenta had an increased capacity (Fig 2). An estimation of HPL in plasma (5) likewise showed a high value (8.8 µg/ml).

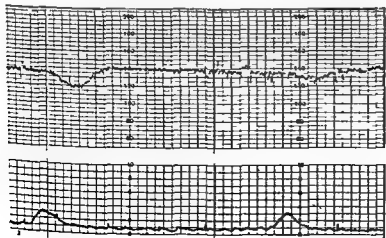


Fig 1 The fetal heart rate pattern in response to uterine contractions



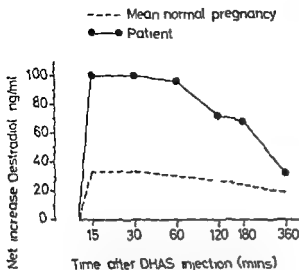


Fig 2 The increase of plasma oestradiol in response to an intravenous injection of 50 mg dehydroepiandrosterone sulphate (DHAS)

### DISCUSSION

A reduced blood supply to the placenta, a common finding in pre eclampsia (3), may cause a decreased nutritive and respiratory function of the placenta, a placental insufficiency. A premature induction of delivery may then be indicated in order to avoid fetal starvation or even death. A combination of low oestriol levels and a positive OCT is most often taken as a sign of placental insufficiency and preterm delivery is recommended regardless of the result of fetal lung maturity tests (4). The present case, however, shows that a disturbed placental function is not a prerequisite for the occurrence of low oestriol levels and a positive OCT. Such findings may be caused by a diseased fetus and thus recorded even in pregnancies with a quite normal placental function.

If estimations of HPL in plasma and/or the DHAS test had been in clinical use, the suspicion of placental insufficiency could have been ruled out in this case.

The DHAS-test is probably safer for assessment of placental function (1) than are estimations of HPL in plasma. The estimation of HPL is, however, more simple to perform than is the DHAS test.

Since the induction of a premature delivery is indicated in cases with placental insufficiency, this diagnosis must be as safe as possible to avoid unnecessary

delivery of premature infants. Low oestriol levels and positive OCT are not conclusive for diagnosing placental insufficiency. These findings should cause suspicion of decreased placental function, a suspicion that can be verified or ruled out by the DHAS test or a measurement of HPL in plasma.

### ACKNOWLEDGEMENT

This study was supported by Stiftelsen Allmänna Barnhusets Minnesfond and the Swedish Medical Council (03495).

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Submitted for publication April 27, 1979

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## CASE REPORT

## MASSIVE EXTRAPERITONEAL BLEEDING — A RARE COMPLICATION OF AMNIOCENTESIS

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Amniocentesis is a widely used procedure infrequently associated with complications. A patient in the 16th week of pregnancy is described in whom amniocentesis for the purpose of genetic counselling was complicated by massive extraperitoneal bleeding into the abdominal wall necessitating the surgical evacuation of a large hematoma. The bleeding most probably resulted from the accidental laceration of the right deep epigastric artery.

Amniocentesis is a procedure widely used in the high-risk obstetric patient near term as well as for mid-trimester artificial abortion and for the purpose of genetic counselling in early pregnancy. The risk attending the procedure is minimal and severe complications are infrequent (3). The incidental perforation of a relatively large blood vessel of the abdominal wall is very rare and may constitute a lethal hazard to the parturient if not recognized. Such a complication is reported occurring during amniocentesis performed for genetic counselling pur-

poses. The skin was noted to be normal. The uterine size corresponded with the dates reported and fetal heart sounds were heard. Hemoglobin was found to be 7.5 g per cent (it had been 10.9 g per cent prior to amniocentesis). The clinical picture was one of severe internal bleeding considered to be an abdominal wall hematoma resulting from amniocentesis. In view of the fact that within hours the swelling extended upwards to the right costal margin and downwards to the symphysis pubis and that the patient's general condition had become worse, she was given a transfusion of 3 pints of blood and transferred to the operating room. Through a right paramedian incision above the pubis a large hematoma of about 1500 ml clotted blood was found upon opening the fascia and was evacuated. The source of bleeding behind the right rectus abdominis muscle was thought to be a perforated right deep inferior epigastric artery. The artery was ligated, the area was drained by a Penrose drain, and the abdominal wall was closed.

The postoperative course was uneventful and the patient was released seven days after the procedure. She was delivered spontaneously of a healthy baby boy in the 39th week of pregnancy.

## COMMENT

During the first half of pregnancy the greatest risk of amniocentesis is the possibility of inducing a traumatic abortion. Maternal complications are infrequent. An abdominal wall hematoma, while mentioned as complicating the procedure, is a rare occurrence indeed. A review of the literature reports only 2 cases of severe hemorrhage among many thousands of amniocenteses performed all over the world (1, 2).

The case presented here indicates that extraperitoneal or subfascial bleeding may be a hazardous even potentially lethal complication.

The artery which was perforated in our case was apparently the right deep inferior epigastric artery which arises from the external iliac artery superior to the inguinal ligament and ascends further, finally be-

## CASE REPORT

A 37-year-old gravida II para I was admitted to the Hasharon Hospital for amniocentesis in the 16th week of pregnancy. In view of the fact that sonar B scan examination had located the placenta in the left lower anterior and lateral wall of the uterus, amniocentesis was performed with difficulty through a right lower-quadrant abdominal tap. Clear amniotic fluid was obtained and sent to the laboratory for genetic studies. One hour after the procedure the patient complained of pain and tenderness over the site of the puncture, which grew rapidly worse. She was pale and complained of increasing weakness. Blood pressure was 100/60, but the pulse rate was 105/minute. Examination and palpation of the abdominal wall revealed a tender bulge in the area of the tap, in the right lower quadrant of the abdomen. The abdomen was soft. Extraperitoneal bleeding was suspected and no ec-

ing located along the abdominis rectus. In order to avoid perforating this artery or its accompanying veins the selection of the site where the needle is introduced for the purpose of amniocentesis is very important. In the second trimester the site preferred is a midpoint between the uterine fundus and the symphysis pubis as close to the midline on the mother's abdomen as possible and as low as possible.

This complication though extremely rare should be borne in mind and evacuation of the hematoma if it continues to increase in size should be done without delay since it may increase the morbidity of the mother even to the point of a severe and possibly fatal outcome.

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*Submitted for publication May 8 1979*

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## CASE REPORT

TREATMENT OF ADVANCED OVARIAN CANCER WITH FIBRINOLYTIC INHIBITOR  
(TRANEXAMIC ACID)

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**Abstract** A patient with inoperable advanced ovarian cancer with metastases and ascites who had received several courses of radiotherapy and chemotherapy is presented. On admission therapy with the fibrinolytic inhibitor tranexamic acid was followed by arrest of ascites and tumor growth. Then an exploratory laparotomy examination of the peritoneum revealed encapsulation of fibrinoid substance proliferation of connective tissue. The patient still receiving tranexamic acid is now free from noteworthy tumor growth.

Treatment of advanced ovarian cancer with ascites is one of the most common major problems of gynecologic oncology. At present three conventional modes of treatment are available: surgery, radiation therapy, and chemotherapy. Regardless of such treatment, tumor recurrence can be expected, and recurrence is often rapid. Therefore, paracentesis, radiotherapy, and chemotherapy are not always effective in controlling ascites in patients with advanced ovarian cancer. On the other hand, malignant ovarian tumors are characterized by both coagulative and fibrinolytic properties, by involving formation of fibrin and conversely by involving degradation of fibrin. Consequently, high levels of fibrinogen/fibrin degradation products (FDP) were demonstrable in the serum as well as in the ascitic fluid in patients with such malignant tumors (1). It was demonstrated experimentally and clinically by Aasted (2, 3) that tranexamic acid, as an inhibitor of plasminogen activation, can prevent the formation and removal of residual fibrin around the tumor and bring about suppression of tumor growth. Treatment with tranexamic acid accordingly gave a favorable outcome with regression of the clinical symptoms and arrest of tumor growth for patients with advanced ovarian cancer as well as breast

cancer. In this report, we describe a case of inoperable advanced ovarian cancer with ascites in which adjuvant

## CASE REPORT

The patient, aged 60-year-old, gravida 3 para 0 was admitted to hospital in April 1977 complaining of persistent abdominal tumor and ascites, probably originating from advanced ovarian cancer stage IV. It was her sixth admission to the hospital. Her present illness had begun with the first admission in 1972, but at that time the patient received linear radiation (3 000 rads) and infusion of colloidal gold ( $^{197}\text{Au}$ ) into the peritoneal cavity because of her inoperable state. Thereafter, temporary remission was obtained, but her clinical symptoms reappeared and further treatment with anticancerous agents such as cyclophosphamide (alkylating agent), 5-FU and mitomycin (OK-432) in addition to repeated paracentesis was tried.

However, signs of growth of tumors did not regress and the interval to recurrence gradually shortened. After a two-month interval from her fifth admission in February 1977, her general condition became worse by reason of distended lower abdominal mass, increased ascites and nutritional disturbance.

The chest film showed no metastatic foci, but remarkable adhesion and large bowel mass were revealed by roentgenogram. The hydroperitoneum of the uterus compressed by tumor mass were also demonstrable by pyelogram. White dilatation as well as congestion were observed by pelvic angiogram.

Coagulative and fibrinolytic studies using blood and ascitic fluid were carried out. PTT was shortened and plasma fibrinogen concentration showed approximately 400 mg/dl. Although the plasminogen concentrations in the plasma and ascitic fluid tended to be extremely low, a rapid increase of FDP titers in ascitic fluid was observed, exceeding 10 µg/ml, and then serum FDP concentrations were also significantly higher than those of benign ovarian tumors (Fig. 1).

On the other hand, inhibitor activity of α<sub>2</sub>-antitrypsin was slightly higher compared to levels of α<sub>2</sub>-macroglobulin and antithrombin.

This paper describes a case of inoperable advanced ovarian cancer with ascites in which adjuvant

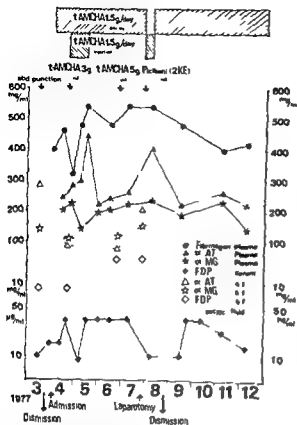


Fig. 1 Fibrinogen, FDP and some fibrinolytic inhibitor levels in a patient with advanced ovarian cancer during an antifibrinolytic therapy.

Thereafter the fibrinolytic inhibitor tranexamic acid [Transamin (t AMCHA) Daichi] was given in a dose of 15–20 g three times a day every day sometimes augmented by infusion of tranexamic acid (3–5 g) into the peritoneal cavity or intravenously.

After 3 months treatment with tranexamic acid the production of ascitic fluid regressed and finally disappeared. Therefore repeated paracentesis was no longer necessary. As a result an outline of abdominal tumor masses previously obscured by ascites became distinct and an exploratory laparotomy was undertaken. At laparotomy strong adhesion between a thickened peritoneum with omentum and the thick wall of an encapsulated ovarian tumor was observed and beneath it large intestinal masses with marked adhesion were also seen. The wall of the ovarian tumor was removed and the adhesions of intestinal masses were repaired as far as possible but most of metastases still remained. Microscopically the tumor mass was confirmed as being metastases of mucous cystadenocarcinoma of the ovary but in some parts of the specimen the tumor cells were surrounded by proliferating connective tissue as well as fibrinoid degeneration (Fig. 2). Furthermore fibrinolytic activity was found around the tumor tissue using a fibrin slide technique. The amount of serum FDP gradually decreased.

The patient felt well and was dismissed from hospital in August 1977. She has been free from noticeable symptoms even though treatment with tranexamic acid was given orally every day.

## DISCUSSION

Ovarian cancer is often associated with effusion. In a series of 61 patients with stage III ovarian cancer, ascites in various degrees was present in 82 per cent (2). Combined treatment with surgery, radiotherapy and chemotherapy is effective in relieving the ascites and in treatment for ascites. Centesis should be avoided because of the loss of an already depleted protein and electrolytes. However, there is no appropriate program for the management of recurrences. Recurrences may often develop at diminishing intervals after an initial course of treatment.

In general it is considered that the fibrinolytic mechanism is depressed in cancer patients. Frequently it is difficult to induce fibrinolysis with cancer which results in the tumor being undigested (7). Furthermore O'Meara suggested that the deposition of fibrin in tumor tissue is essential for the growth and infiltration of tumor cells and the clotting factors in ascitic fluid found to be higher in the presence of malignant tumors (1).

Malignant ovarian tumors, on the other hand, possess coagulative and fibrinolytic properties reflected in the occurrence of FDP in 72 per cent of the sera of such tumor patients (8) and in high concentrations in ascitic fluid (5). We have a data to show that FDP levels in ascitic fluid of ovarian cancer are extremely high (4). In addition, no ovarian cells and benign ovarian tumor cells in culture showed low FDP levels, whereas malignant ovarian tumor cells displayed high FDP levels. In view of these findings, Åstedt *et al.* (9, 10) proposed a new treatment for advanced malignant tumors: coagulant therapy to prevent fibrin deposition, combined with antifibrinolytic therapy to prevent lysis of fibrin already formed. This might be more effectively with tumor growth. They reported that adjuvant therapy with heparin and tranexamic acid has brought about an inhibition of tumor growth in two patients with advanced cancer as well as ovarian cancer.

Although our patient with inoperable advanced ovarian cancer and ascites was treated successfully



Fig 2 Island of tumor cells surrounded by vacuolation and proliferation of connective tissue and fibrinoid degeneration (PAS  $\times$  00)

radiotherapy and chemotherapy her clinical symptoms reappeared at a shorter duration. For her recurrence single treatment with tranexamic acid was given. As a result the patient has responded well to such therapy and has been free from her recurrent clinical symptoms despite the existence of residual tumors.

It remains however to determine whether these results can be ascribed solely to the antifibrinolytic therapy because the patient has received radiotherapy and chemotherapy for many years. Nevertheless this report suggests that antifibrinolytic therapy with tranexamic acid has a direct effect on regression of tumors and arrest of tumor growth in the patient with advanced ovarian cancer as reported by Åstedt

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## ACKNOWLEDGEMENTS

This study was supported by grant from the Cancer Center, Tokyo Medical College. We wish to thank Professor S. Soder and Professor B. Åstedt, University of Lund, for their kind critical review of this paper.

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Submitted for publication January 29 1978

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## ANNOUNCEMENT

The International Federation of Gynecology and Obstetrics  
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## CLINICAL FETAL MONITORING

### The usage and relationship to trends in cesarean delivery and perinatal mortality

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During the six years 1970–1975 62 266 deliveries were performed at Women's Hospital Los Angeles County University of Southern California Medical Center. Of 106 (29 per cent) were monitored with an incidence of 18 per cent in 1970 which rose to 35 per cent in 1975.

The patients were divided into monitored and unmonitored groups for comparison of cesarean section rates. Various aspects of perinatal mortality. The overall cesarean section rate was 9.6 per cent with 2 830 cesareans performed in monitored patients (16 per cent) and 3 124 in unmonitored a 7 per cent incidence including repeat cesarean. The cesarean rate remained remarkably stable during the 6-year study period even though the monitoring incidence nearly doubled. From 1970 to 1975 the intrapartum fetal rate fell progressively in contrast to the incidence of intrapartum fetal deaths which remained unchanged at all 50 per cent survival rate was achieved in the birth weight range of 1 200 grams. A particular group of fetal patients who apparently benefited from intrapartum monitoring were those liveborns with birth weights of 1 000 grams or less. Over the study period the neonatal mortality in monitored patients declined whereas mortality in neonates who were not monitored during labor remained high.

The introduction and rapid acceptance of electronic fetal monitoring in obstetrical practice is self-evident. Simplified techniques and improved instrumentation application has been expanded to include intrapartum heart rate testing (2 7 13 16) and the employment of total intrapartum monitoring in many institutions. The question of applicability of intrapartum monitoring in the high risk patient is now raised and a number of current studies seek to assess the impact when monitoring is attempted in all patients (3 14).

Although two prospective studies have attempted to define the role of intrapartum monitoring in high risk pregnancy the benefits and liabilities associated with monitoring have not been unequivocally quantified (5 11). Documentation of complications is

usually in the form of case reports or retrospective review in which liability is only partially revealed (1 4 6 17). Likewise evidence of benefit is difficult to isolate and measure since concurrent obstetrical procedures are changing as a result of increased interest and an enlightened approach to perinatal care (9 12 15).

At present it is questionable if a prospective controlled scientific study can be accomplished since it is most difficult to singularly isolate the process of electronic monitoring and as the mounting indirect evidence of benefits raises the ethical questions whether it should be attempted (8). In addition the endpoints judging success such as Apgar scores and acid base are subject to many variable factors. The endpoint of intrapartum death although an absolute as shown in this paper is very infrequent thus making it an impractical basis for comparison in a study with limited numbers of patients.

In view of the expense need for increased patient surveillance and the educational demand imposed it is not unreasonable that accumulating evidence regarding the role of fetal monitoring be examined (8 10). Unfortunately endpoints which seek to measure pregnancy outcome such as Apgar score or neonatal acid base status are not uniformly predictive of long term condition. A clearly measurable undisputable endpoint is perinatal death. It is recognized that a more ideal critical evaluation would be the assessment of neonatal morbidity CNS sequelae or long term infant follow up. This approach unfortunately was not possible within the scope of this study. This report describes the increasing usage of monitoring in a large obstetrical service and explores its possible effect on the cesarean section rate. The overall trend in perinatal mortality is evaluated and perinatal outcome is compared in large monitored and unmonitored groups.



**Table 1** The yearly incidence of cesarean deliveries is shown with the per cent in parenthesis. Cesarean in monitored patients stabilized at roughly 15 per cent after a significantly higher incidence when monitoring was introduced in 1970

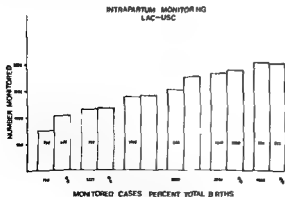
	Cesarean section at LAC/USC 1970-1975					
	1970	1971	1972	1973	1974	1975
Deliveries	9 775	9 425	9 421	10 434	11 585	11 625
Cesareans	910 (9.3)	872 (8.7)	910 (9.6)	1 068 (10.2)	1 078 (9.3)	1 166 (10.0)
Primary	630 (6.4)	573 (6.1)	617 (6.5)	753 (7.2)	763 (6.7)	801 (6.9)
Repeat	280 (2.9)	249 (2.6)	293 (3.1)	315 (3.0)	315 (2.7)	365 (3.1)
Monitored patients	1 798 (18)	2 337 (25)	2 828 (30)	3 329 (32)	3 476 (33)	4 068 (35)
C/S in monitored patients	354 (20)	360 (15)	465 (16)	539 (16)	543 (14)	575 (14)

## MATERIAL AND METHODS

During the six years 1970-1975 62 266 births occurred at Women's Hospital Los Angeles County-University of Southern California Medical Center (LAC/USC). Intrapartum monitoring was instituted on a selective basis in the last three months of 1969 with 10 per cent of patients monitored. Due to the large patient volume, limitations of available instrumentation and lack of trained personnel, an attempt to monitor all patients was not undertaken and is not currently feasible. However, an increasing attempt was made to monitor all patients with high risk conditions during the years 1973-74 and since then the incidence of monitoring has remained roughly one third of all patients being monitored even though the total number of patients monitored has continued to increase. In this selective approach, the most common indications for monitoring were poor progress in labor, oxytocin administration for augmentation or induction of labor, meconium passage, hypertensive disorders of pregnancy and abnormal fetal heart tones by auscultation. Other common indications included breech presentation, maternal medical disorders such as diabetes mellitus and obstetrical complications such as abruptio placentae.

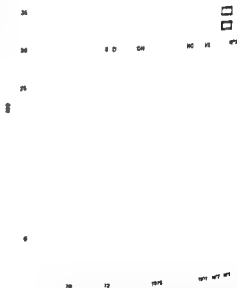
The monitored group was thus generally composed of patients with high risk conditions while the unmonitored group consisted of patients considered to be low risk. However, it is clear that an absolute definition and categorization of these groups is not possible since monitoring has been contraindicated in some high risk patients whose risk condition went unrecognized. Conversely, patients in the monitored group were no doubt at times with minimal risk indications.

A patient was placed in the unmonitored group if 20 consecutive minutes of interpretable fetal heart rate (FHR) was obtained in order to eliminate cases of



**Fig 1** The number of patients monitored increased in each six month interval over six years. The relative incidence of monitoring was stable during 1973 through 1975 in spite of a larger total number as a result of more births.

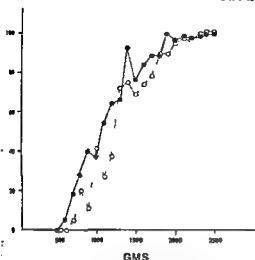
## PERINATAL MORTALITY LAC/USC 1970-1975



**Fig 2** Perinatal mortality rates progressively declined during the years 1970-1975. The yearly perinatal mortality rate defined by the World Health Organization (WHO) for the United States is contrasted. The largest reduction was in neonatal mortality.

## NEONATAL SURVIVAL

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● 1970-72  
○ 1973-75

3 The neonatal survival in infants weighing  $\leq 2500$  g shown in a three year comparison. An infant has a 50 per cent chance of survival with a birth weight of 1200 g.

minimal FHR data which would not likely have served as a guide in clinical management. The FHR UC data was obtained by direct methods using a fetal electrode and trans-cervical intrauterine catheter in excess of 95 per cent of patients. Indirect monitoring methods were not available in the early years of the study period and at present are generally used only when direct methods are contraindicated as in breech presentation, transverse lie and suspected early labor. Indirect monitoring was introduced in 1970 and has been extremely useful when abnormal or confusing FHR tracings are encountered. This supplementary information is currently obtained in about 10 per cent of patients being monitored electronically.

During the years studied, all neonatal and fetal deaths were evaluated. Fetal deaths were subdivided into two groups — those occurring antepartum and intrapartum. No fetal benefits could be anticipated from intrapartum monitoring when death had occurred prior to admission. An intrapartum death was therefore arbitrarily defined as a fetal loss occurring after the positive identification of fetal heart tones or viability on admission to the labor room. Thus probable labor related deaths such as those seen with abruptio placentae, prolapsed cord, etc. were classified as antepartum deaths whenever the fetal heart activity was absent on admission.

## RESULTS

**Monitoring Usage.** A total of 18106 of the 62266 births, or 29 per cent of all patients were monitored in some way. Thus patients with an antepartum fetal death were included in this overall total

Table II Overall fetal deaths at LAC/USC 1970-1975 are presented. There are approximately four antepartum deaths for each intrapartum death. The number in parenthesis is the rate per thousand.

		Fetal deaths 1970-1975	
		Number	
Total deliveries		62 266	
Fetal deaths	(14)	856	
Antepartum	(11)	701	
Intrapartum	(3)	155	
Weight $< 1500$ g		360	
Antepartum		276	
Intrapartum		84	
Weight 1500-2500 g		238	
Antepartum		212	
Intrapartum		26	
Weight $> 2500$ g		258	
Antepartum		213	
Intrapartum		45	

even when only uterine activity was monitored. The incidence of monitoring rose from 18 per cent in 1970 to 35 per cent in 1975. The incidence of monitoring plateaued during the final three years, although the total number of patients monitored continued to increase as shown in Fig 1.

**Cesarean Delivery.** Of the 62266 births, 5954 (9.6 per cent) were by cesarean section. Of these, 4137 (6.6 per cent) were primary and 1817 (2.9 per cent) were repeat cesarean sections. The yearly incidence is given in Table I and although absolute numbers increased, one will note the relatively stable incidence of this type of delivery over the five years.

In general, monitoring was used only in patients who had a primary cesarean delivery and not in those undergoing repeat sections. A total of 2830 cesareans were done in the 18106 monitored patients, or an overall incidence of 16 per cent. The highest incidence of cesarean section in monitored patients, 20 per cent, occurred in 1970 with the introduction of this technique. Over the subsequent five years, the mean incidence was 15 per cent. In the unmonitored group of 44160 patients, there were 3124 cesareans which included 1817 repeat sections, an overall incidence of 7.1 per cent. When repeat cesareans were eliminated from consideration in the unmonitored group, there were 1307 sections in 42343 patients, an incidence of 3.1 per cent.

Table III The decreasing trend in fetal deaths is shown for both antepartum and intrapartum groups. The decline in incidence of occurrence is most significant when intrapartum deaths in 1970 are compared to those in 1975. The occurrence of antepartum deaths remained relatively unchanged

	Fetal deaths at LAC/USC						Total
	1970	1971	1972	1973	1974	1975	
Alive on admission	9 654	9 303	9 318	10 318	11 475	11 497	61 96
Antepartum fetal deaths	121 <sup>†</sup>	122	103	116	110	116 <sup>†</sup>	688
Intrapartum fetal deaths	36	33	34	0	16	16	159
Intrapartum > 1500 g	18	14	17	7	9	6	71

Comparison 1970 vs 1975 <sup>†</sup> Not significant  $p < 0.005$

**Perinatal Mortality** There were 856 fetal deaths and 735 neonatal deaths during the six year period for an overall perinatal mortality rate of 26/1000. The perinatal mortality rate for the five year period preceding this study 1965–1969 was 49/1000. The progressive yearly declines during the six years of this study are shown in Fig 2 with the lowest rate occurring in 1975. One will note that the major reason for the fall in perinatal mortality was the reduction in neonatal mortality.

**A Fetal Death** Of the 856 fetal deaths 701 occurred antepartum and 155 during the intrapartum period as defined previously. The occurrence of these deaths by weight group is presented in Table II.

The yearly data which document the decreasing incidence of fetal death is given in Table III. It is of interest to note that when 1970 is compared with 1975 the overall intrapartum death rate fell from 7/1 000 to 1.4/1 000 or 62 per cent whereas the antepartum rate only decreased 13 per cent. The significant decrease in occurrence of intrapartum death is even more dramatic when only infants weighing more than 1 500 grams are considered. In this group the rate fell from 1.8/1 000 to 0.5/1 000 a 72 per cent decline.

**B Neonatal Death** Neonatal death rates successive year as shown in the lower area of Fig 2. Of the 735 total occurred in 868 infants (1.4 per cent of total births) with birth weights of 1 500 grams or less. Newborns that weighed 1 500 grams or less than counted for 61 per cent of the overall mortality.

A 50 per cent survival rate was achieved weight range of 1 200–1 300 grams and 3.

**Monitored vs Unmonitored Patients** Considered in monitored and unmonitored the intrapartum and neonatal death rates. The 701 patients with antepartum fetal death not considered in either group. A patient in the monitored group only when 20 minutes of data was obtained for interpretation and clinical. For this comparison a patient was classified unmonitored in cases where only 1 pressure was measured.

**A Intrapartum Fetal Deaths** The comparison of intrapartum fetal death in the monitored and unmonitored groups is shown in Table IV. The death rate in the monitored group shown in the upper portion

Table IV A Overall monitored versus unmonitored intrapartum fetal deaths at LAC/USC 1970–1975 are shown

	Intrapartum fetal deaths	
	High risk monitored	Low risk unmonitored
Total patients	17 363	44 202
Fetal deaths	24	131
Rate per 1 000	1.4	1.3

Table IV B Monitored versus unmonitored intrapartum fetal deaths at LAC/USC 1970–1975 the corrected comparison in weights over 1

	Intrapartum fetal deaths	
	High risk monitored	Low risk unmonitored
Total patients	17 089	43 54
Fetal deaths	16	15
Rate per 1 000	0.9	1.3

Table V Intrapartum deaths in weight > 1500 g (at LAC/USC 1970-1975) occur less often in monitored patients. Patients with severe congenital anomalies or birth trauma associated with death are eliminated in both groups

	Intrapartum fetal deaths	
	High risk monitored	Low risk unmonitored
Total patients	17 089	43 524
Fetal deaths	7	36
Rate per 1 000	0.4	0.8

0.01  $p < 0.1$

significantly lower than the unmonitored group ( $p < 0.005$ ) with the chi square test. However, evaluation of only these data would be misleading as one must also examine these deaths. In the unmonitored group 76 of the 131 intrapartum deaths were in very low weight fetuses (1 500 grams) whereas only 8 of 24 deaths in monitored patients were similarly very low weight. The unmonitored group thus contained a disproportionately large number of fetal deaths in very low weight fetuses. A factor which might explain this finding is that monitoring was used infrequently when the fetus was written off or felt to be unsalvageable, particularly during the early years of this study.

In order to eliminate this factor, it was decided to analyze fetal deaths in which birth weights exceeded 1 500 grams. There was a total of 71 such deaths with 33 in the monitored group and 55 in the unmonitored

Table VI The neonatal mortality rate at LAC/USC 1970-1975 is compared in monitored and unmonitored groups. A similar rate is found in weights over 1 500 g

	Neonatal mortality	
	Monitored	Unmonitored
Total live births	17 339	44 071
Neonatal deaths	181	554
Rate per 1 000	10.4	12.6
> 1 500 g		
Total live births	17 073	43 469
Neonatal deaths	90	198
Rate per 1 000	5.3	4.6

0.05 difference not significant

Table VII The neonatal mortality (at LAC/USC 1970-1975) in infants weighing less than 1 500 g is significantly lower in the monitored group. This difference is most marked in infants weighing 1 000 to 1 500 g

	Neonatal mortality	
	Monitored	Unmonitored
≤ 1 500 g		
Live births	266	602
Neonatal deaths	91	356
Rate per 1 000	34.2	59.1
1 000 - 1 500 g**		
Live births	203	363
Neonatal deaths	54	147
Rate per 1 000	26.6	40.5
< 1 000 grams**		
Live births	83	239
Neonatal deaths	37	209
Rate per 1 000	58.7	87.4

$\chi^2 = 15.8$   $p < 0.005$   $\chi^2 = 5.39$   $p < 0.025$   $\chi^2 = 3.06$   $p < 0.1$

group as seen in the lower portion of Table IV. A number of these intrapartum losses were associated with recognized severe congenital anomalies or birth trauma in which monitoring per se could not have changed outcome. Therefore, both groups were similarly corrected for problems such as anencephaly, hydrocephaly with decompression, severe shoulder dystocia of more than five minutes, and a trapped breech of more than 20 minutes duration.

The corrected fetal death rates are presented in Table V and the difference in these rates does not achieve statistical significance but is suggested ( $p > 0.05 - 0.1$ ).

II Neonatal Deaths. The overall neonatal mortality rates for the monitored and unmonitored groups are presented in Table VI. Although the overall mortality is strikingly different, the death rates are comparable in infants weighing more than 1 500 grams. This finding could be anticipated since the previously stated factors used as indications for fetal monitoring are known to be associated with an increased mortality. Since the mortality rates in infants > 1 500 grams are similar, the overall differences seen in the total rates must occur in those newborns weighing ≤ 1 500 grams. The comparative data from these neonates is given in Table VII. The overall newborn mortality of the monitored group weighing 1 500 grams or less is significantly lower than in the unmonitored group. Distribution of neonatal weights was examined and found to be randomly distributed in both groups. The

Table VIII The yearly comparisons of neonatal mortality (at LAC/USC 1970-1975) in monitored and unmonitored neonates  $\leq 1500$  g is given. The most dramatic differences are apparent in 1974 and 1975

	Monitored			Unmonitored		
	Births	Deaths	Mortality %	Births	Deaths	Mortality %
1970	13	5	38.5	127	86	67.7
1971	26	9	34.6	127	80	63.0
1972	34	18	52.9	108	56	51.9
1973	39	20	51.3	86	43	50.0
1974	63	16	25.3	84	52	61.9
1975	91	23	25.2	70	39	55.7
Total	266	91	34.2	602	356	59.1

yearly increases of monitoring usage in small fetuses is apparent in Table VIII. The highest mortality rate occurred in 1972 and subsequently has dramatically declined as experience was gained in this type of patient and as more were monitored. In contrast mortality in the unmonitored group has remained virtually unchanged over the six years.

### COMMENT

During the six years evaluated there was a progressive downward trend in perinatal mortality. Multiple factors which no doubt contributed to improved outcome were more comprehensive management of complicated medical obstetrical problems, improved obstetrical anesthesia, vigorous newborn resuscitative procedures, and a broadened neonatal intensive care capability.

Other factors which also exerted their impact during this time were a liberalized approach to abortion, a fall in the incidence of low birth weight births from 9.4 per cent to 7.4 per cent, a trend toward cesarean delivery in breech presentation and the introduction of continuous methods of intrapartum monitoring. In view of the many factors associated with a declining perinatal mortality it is impossible to state a direct causal role relating only to intrapartum monitoring.

The question is often raised: does the introduction and usage of clinical monitoring cause the cesarean section rate to rise? Although there was an initial higher incidence of cesarean delivery in monitored patients in 1970 (20 per cent), the cesarean rate over this six year study stayed remarkably stable. The finding of an originally higher incidence of cesarean delivery is commonly reported and may be the result of anxiety with overreaction to the new information available. However, in our experience, stability in the

rate occurred over the next five years, although the incidence of monitoring nearly doubled. As expected with selective high risk indications used for monitoring, the incidence of cesarean section was higher in monitored patients than in unmonitored patients.

The data regarding overall fetal death does not alter our comment. First, antepartum deaths were 6 times more common than those occurring during labor. The need for better screening methods to identify such patients and estimate fetal well-being is urgent. An additional factor possibly reflecting minimal reduction in rate is caused by our patient population which frequently receives little or no prenatal care, and because we are a center to which patients with antepartum death are referred. Surely monitoring will not eliminate intrapartum death, although its occurrence will be reduced to an extremely low incidence. In a critical review of the 18 monitored deaths, there were suggestive fetal FHR patterns in six instances, which if acted upon more rapidly, may have prevented death. Thus, in this monitored group of over 18,000 patients, only one unexpected death occurred which was associated with fetal arrhythmia.

As one attempts to evaluate the effect of monitoring on outcome, an obvious weakness is the comparison of dissimilar groups. However, the monitored group composed of high risk patients may have contributed more heavily to overall mortality, in fact the perinatal mortality in this high risk group was lower than in the unmonitored group. In addition to the lower incidence of intrapartum deaths, perinatal patients who as a group benefited from intrapartum monitoring were those less than 1500 grams or less. This fact in particular tends to negate the argument that monitoring merely prevents fetal loss and shifts the

of death into the neonatal period. In comparing monitored and unmonitored groups it is apparent the presumed normal or unmonitored group contributes significantly to perinatal losses. In order to minimize mortality and enhance intrapartum care would seem not unreasonable to extend monitoring to most patients during the labor process. During the six years of study a different philosophy emerged regarding the care of the very small fetus. With the availability of expert neonatal intensive care and as a result of accumulating experience any fetus judged to weigh 1 000 grams or more considered fetal intensive care and would be promptly delivered by cesarean section should non-correctable fetal distress be encountered. The term 'promptly delivered' is chosen since abnormal FHR patterns seem to evolve more rapidly in the small fetus reflecting a diminished tolerance. This liberalized approach coupled with more small fetuses being monitored has met with gratifying neonatal results. The fetuses weighing between 500-1 000 g are being more frequently monitored even though operative intervention is ruled out in an attempt to correct and minimize possible deleterious effects during labor should the fetus survive. The 1 000 g weight selected is arbitrary and may be revised with continuing experience and when better determination of weight is possible. The monitored fetus apparently enjoys an enhanced survival over his unmonitored counterpart. In addition to mere survival long term benefits may include prevention of brain injury since fetal damage at birth of death should also be avoidable. The desirability and need for a well-designed perinatal study of long term follow up to further evaluate the role of clinical monitoring is self-evident.

## ACKNOWLEDGEMENT

The authors wish to express their gratitude to Richard I. Paul M.D. for his statistical evaluation of the data.

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Submitted October 10 1978

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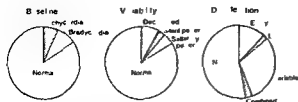


Fig 1 The frequency of the various FHR changes during the first stage of labor in the total material (percentage)  $n=2566$

per cent VE 1.4 per cent forceps). The uncorrected perinatal mortality was 0.69 per cent. No infants died during delivery.

Table I and Fig 1 show the frequency of various FHR changes in the total material. Deviations in basal frequency and variability occurred in about 15 per cent whereas different types of decelerations specially variable or early could be shown in almost 50 per cent of all recordings. Tachycardia, silent pattern or late decelerations occurred rarely. The tracing was completely normal in 40 per cent of all deliveries.

Table I and Fig 2 show the frequency of various FHR changes in those newborns that had an Apgar score less than 7 at 1 minute. In this group tachycardia during delivery was considerably more common (26 per cent) than in the total material. On the other hand the frequency of bradycardia was not appreciably increased. In the total material silent pattern occurred in only 2.5 per cent but here the proportion had increased to 22 per cent. The frequency of late and combined decelerations was also increased to 9 and 11 per cent respectively. Only 8 out of 116 newborns in this group had a completely normal FHR recording during the first stage of labor.

Table I and Fig 3 list the corresponding figure of newborns with an Apgar score less than 7 at 5 minutes. During 1977 this group included 29 newborns

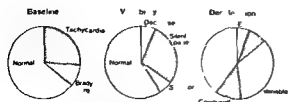


Fig 2 The frequency of the various FHR changes at those deliveries where the newborns had an Apgar score <7 at 1 minute (percentage)  $n=116$

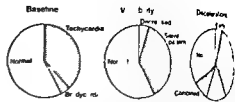


Fig 3 The frequency of the various FHR changes at those deliveries where the newborns had an Apgar score <7 at 5 minutes (percentage)  $n=21$

(0.9 per cent of all births) of whom 21 were recorded during the first stage of labor. Tachycardia, silent pattern or late decelerations were the dominant FHR changes in this group and had already occurred during the first stage of labor in 18 out of 21 deliveries. Bradycardia, reduced variability and early decelerations were only exceptionally noted. Also the frequency of variable decelerations was reduced in this group.

Fig 4 shows the percentage of newborns with an Apgar score less than 7 at 1 and 5 minutes at various types of FHR changes. About 20 per cent of those that during the first stage of labor had late or combined decelerations had reduced Apgar score at 1 minute. For silent pattern this was almost doubled (39 per cent). At 5 minutes 19 per cent of all newborns with tachycardia and decelerations had an Apgar score less than 7, corresponding percentage for newborns with silent pattern or late decelerations were 12.5 and 16.1 per cent respectively.

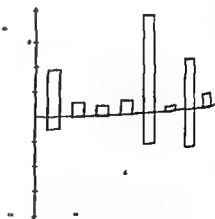
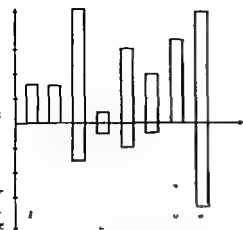


Fig 4 Percentage of newborns with an Apgar score <7 at 1 and 5 minutes at various types of FHR changes. For example 19 per cent of all newborns with tachycardia and decelerations had an Apgar score <7 at 1 minute and 5 per cent at 5 minutes.



Percentage of newborns with an Apgar score <7 at 1 minute in combinations with tachycardia and other changes

tain combinations of the above mentioned changes revealed an increased percentage of reduced Apgar score (Fig 5 and 6). Combinations of tachycardia, silent pattern and decelerations were found in almost half of the cases together with reduced Apgar score at 1 minute. The same tendency could be found at 5 minutes.

## DISCUSSION

At maternity clinics report reduced perinatal mortality after introducing electronic fetal monitoring and pH analysis of the fetal scalp blood (2, 7, 4). The main reason for the reduction of fetal death during labor was considerably reduced. Whether morbidity was also reduced is so far unclear, but recently (15) demonstrated a reduced frequency of injury and also less need for neonatal intensive care of newborns monitored with CTG and pH during delivery.

The present investigation used fetal electronic monitoring routinely in an unselected delivery material. The frequency of various FHR changes is compared with other published material, but this is due to other authors not having solely studied changes during the first stage of labor or mainly high risk pregnancies. Of the 116 newborns found to have an Apgar score less than 7 at 1 minute, 101 during the first stage of labor showed one or more of the following FHR changes: tachycardia, silent pattern, late and combined and variable decelerations. The corresponding figure for

newborns with an Apgar score less than 7 at 5 minutes is 11 out of 21.

Of the newborns with reduced Apgar score only 8 of 116 at 1 minute and none of 21 at 5 minutes had a normal FHR pattern.

This indicates that electronic fetal heart rate monitoring provides possibilities to suspect the diagnosis of fetal distress already during the first stage of labor, despite the fact that the course of events during the second stage of labor have not been evaluated in this study.

However, the risk of overdiagnosing fetal distress by CTG is considerable, the number of falsely ominous FHR recordings is relatively high (1). This is also noted in this material, where the majority of infants with tachycardia, silent pattern, late and combined decelerations had a normal Apgar score (Fig 4). If several of the FHR changes occurred at the same time in the same patient, however, the proportion of reduced Apgar score considerably increased (Figs 5 and 6).

In order to establish the distress diagnosis, several studies have shown the value of pH analysis of fetal scalp blood (10, 15). This is illustrated by deliveries in this material where late decelerations appeared. During the majority of these deliveries, pH analysis was repeatedly made, in fully half of the cases a normal acid base balance could be demonstrated. In 60 per cent of these cases of vaginal delivery could be performed.

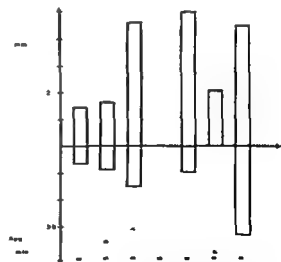


Fig 6 Percentage of newborns with an Apgar score <7 at 1 and 5 minutes in combinations with silent pattern and other FHR changes



In the present study no intrapartum fetal deaths occurred which stress the postulate that these deaths can be reduced with intensive fetal monitoring (9)

The FHR changes in this material have been related to Apgar score as an assessment of the newborns condition. The newborns condition could not be related to pH analysis of cord blood as the number of these measurements were too small. However, the low perinatal mortality and the low frequency of newborns with an Apgar score less than 7 at 1 minute (4.5 per cent) and 5 minutes (0.8 per cent) may suggest the benefit of intensive fetal monitoring. Hochuli *et al* (7) in a review article found a better fetal outcome of newborns who had been monitored during labor and delivery. However, to which categories of patients fetal monitoring should be restricted, if a restriction shall be made, seems unsettled (5).

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*Submitted for publication February 14 1979*

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# SELECTIVE VERSUS ROUTINE INTRAPARTUM MONITORING COMPARISON OF EFFECTS ON PERINATAL OUTCOME

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This study compares perinatal results of 7 604 de-  
liveries in two successive years (1976-1977) in which no  
changes occurred other than a four fold increase in  
intrapartum monitoring.

In the first year 15.4 per cent of births were monitored  
internally. In the second year all births were monitored  
internally. The exception of patients admitted at an advanced stage  
of labor and elective cesarean section. The results show no  
significant improvement either in intrapartum or in early  
neonatal mortality rates. The same results obtained in cesar-  
ean section rate and in instrumental deliveries. The only  
significant result is a significant reduction of low Apgar scores  
in the unselected monitoring group (1977). The low mor-  
tality is considered to be a result not only of increased mon-  
itoring but also of active management of labor and of a short  
duration of labor and of intensive neonatal care.

The quality of obstetrical care is currently evaluated  
by perinatal indices i.e. perinatal mortality and  
perinatal morbidity. While the contribution of intra-  
partum electronic monitoring of labor in reducing  
perinatal mortality is well established in high risk de-  
liveries (9 11 12 13 17) its effect on perinatal mor-  
bidity and neonatal morbidity in low risk deliveries  
has not been clearly defined.

Monitoring on intrapartum fetal monitoring may be  
divided into two groups: the first concerning selec-  
tive monitoring of high risk cases (9 11 12 13 17)  
and the second the routine monitoring of all deliveries  
(5 6 10 14). In the first group the perinatal in-  
dices of monitored high risk cases cannot be com-  
pared with those of unmonitored low risk patients  
because of fundamental differences independent of  
the monitored or unmonitored status. In the second  
group of studies perinatal results for a period in  
which more or less universal monitoring was used are  
compared with results recorded in the same institu-  
tion in premonitoring years. The problem in this  
study is that changes other than monitoring  
occurred during those years which undoubtedly

contributed to improved fetal results such as early  
identification and intensive prenatal care of high risk  
cases improved management of medical-obstetrical  
problems higher cesarean section rate better obstet-  
ric anesthesia and improved neonatal care. Elimina-  
tion of this bias is a difficult task.

The present study deals with perinatal results in  
two successive years (1976-1977) in which no policy  
changes occurred other than a four fold increase in  
electronic monitoring of labor.

## MATERIAL AND METHODS

During the years 1976-1977 there were 7 604 deliveries in  
the Department of Obstetrics A of the Hakurya Materni-  
ty Hospital Tel Aviv. The number of newborns weighing  
1 000 grams (g) or more was 7 678. In the first year 15.4 per  
cent of all births were monitored internally. The monitored  
births comprised the high risk (HR) cases as well as the low  
risk (LR) cases where obstetrical complications developed  
during labor. At the end of 1976 more monitors arrived.  
From the beginning of 1977 all births both HR and LR  
were monitored internally with the exception of patients  
admitted at an advanced stage of labor and elective cesarean  
section. The average percentage of monitoring rose to 33  
per cent in 1977.

With few exceptions all our patients get satisfactory  
antenatal treatment. The obstetrical risk is evaluated within  
the fifth month of pregnancy and each pregnant woman is  
scored and classified as HR or LR. The HR group gets in-  
tensive antenatal treatment in an HR clinic. The LR patients  
get routine treatment as long as they do not develop any  
complication. If a complication does develop the patient  
becomes HR and is treated in an HR clinic.

Apart from the higher percentage of monitoring in the  
year 1977 there have been no changes in the treatment of  
parturients which included changing the position of the pa-  
tient the administration of oxytocin oxygen meperidine  
and promethazine paracervical pudendal and epidural  
blocks. There is at least one obstetrician in the delivery  
room 24 hours a day. The Chief resident a neonatologist  
and an anesthetist are always available. This team is well  
trained in intensive resuscitation of the newborn.

Table I Perinatal death (birthweight  $\geq 1\ 000$  g) in selective versus routine intrapartum monitoring

	Number of deliveries	Number of births	BPMR	ENMR†	No. of stillbirths $\geq 1\ 000$ g		
					Ante partum	Intra partum	Total
Selective monitoring 1976	4 065	4 090	12.2	4.7	23	8	31
Routine monitoring 1977	3 539	3 538	11.3	5.4	14	7	21

BPMR = Basic perinatal mortality rate (expressed per 1 000 births) † ENMR = Early neonatal mortality rate (expressed per 1 000 live-

Vaginal delivery is permitted in breech presentation if the parturient has already had a vaginal delivery of a full term baby and after performing an abdominal roentgen ray to assess the size of the fetus and to rule out malformations and hyperextension of the head (2). An additional criterion for vaginal delivery in primiparae is a normal pelvimetry according to Colcher Sussman (3).

Active management of labor is the method adopted in our department, e.g. early amniotomy and intravenous administration of oxytocin in progressive doses until an optimal uterine activity of 276 Montevideo units in primiparae and 220 in multiparae is reached (15–18). Application of monitors and tracing interpretation is done by the obstetrician, as are the assisted births. In all births the partogram, the method of delivery, Apgar at one and five minutes and the monitoring are registered. The perinatal results were checked and compared for the years 1976 and 1977, the years of selective and routine monitoring respectively.

## RESULTS

The basic perinatal mortality rate (BPMR) is defined according to the recommendations of the 7th General Assembly of the International Federation of Gynecology and Obstetrics as the number of deaths occurring before birth or less than 168 hours after birth per thousand births of 1 000 g or more. During the select-

ed monitoring period (1976) BPMR was 50.4 (12.2/1 000). In the routine monitoring period (1977) BPMR was 40/3 538 or 11.3/1 000 (Table I). This difference is statistically not significant ( $p > 0.05$ ).

Among the 31 stillbirths recorded in 1976, 11 were intrapartum deaths (Table I). Three stillborns weighed 1 000–1 050 g. Though death was attributed to these cases, cesarean section was performed because of their prematurity. The fourth death was the result of impaction of the shoulder in a fetus weighing 3 850 g. In the fifth case fetal death occurred during the 10–15 minutes between admission to the delivery room (registration, shaving and giving an enema) and transfer to delivery room. The sixth death was of a omphalocele fetus accompanied by hydramnion. The seventh death was the result of neglected labor and misjudgement in the estimation of gestational age and of the fetal weight, which was 1 740 g, as assessed to be about 1 000 g. The eighth death was of a fetus with placenta velamentosa with ruptured previa. The fetal death was immediate.

In the routine monitored group (1977) seven of the intrapartum deaths were recorded. Four of these were anencephalic. One had hydrocephalus, weighing 1 090 g, had prolapse of cord. The other case was a fetus small for gestational age, weighing 2 500 g, which died a few minutes after admission to the delivery room.

The early neonatal mortality rate (ENMR) is defined as the number of early neonatal deaths occurring less than 168 hours from the time of birth per thousand livebirths of infants weighing 1 000 g or more. During the period of selected intrapartum monitoring (1976) the ENMR was 4.6 compared to 5.4 in the routine monitoring period (1977) (Table I). In both periods the majority of neonatal deaths were due to malformations and respiratory distress. As shown in Table II, the deaths were quite similar in the monitored or unmonitored status. If the differences in ENMR between the two periods are statistically insignificant ( $p > 0.05$ ).

Table II Number of early neonatal deaths

Diseases	Selective monitoring	Routine monitoring†
Respiratory distress	8	5
Malformations	3	7
Trisomy	3	—
Diaphragmatic hernia	2	—
Sepsis	—	2
Potter's syndrome	—	2
Anencephaly	1	—
Hydrops fetalis	1	—
Others	1	3
Total	19	19

1976 † 1977

to the potential complications of perforation of the skull by internal monitoring not a single case of infection has been documented. Special attention was paid to fetal scalp injuries or infections caused by the scalp electrode. No significant injuries or infections have been encountered among the newborns since the introduction in 1974 of the automatic-raft scalp electrode in place of the traumatic electrode.

The cesarean section rate rose from 4.5 per cent in 1970 to 4.8 per cent in 1977. The instrumental delivery rate rose from 9.2 per cent to 10.5 per cent. However, neither the change in cesarean section rate nor the instrumental delivery rate is statistically significant ( $p > 0.05$ ).

Neonatal morbidity directly related to obstetric causes has been evaluated by the percentage of 1-minute Apgar scores of 6 or less. In the routinely monitored group the rate was 0.96 per cent (34 cases) and in the selectively monitored group 1.66 per cent (6 cases). The difference is statistically significant ( $p < 0.01$ ).

## DISCUSSION

It has been established that the intrapartum monitoring of patients has a favorable effect on perinatal outcome.

This conclusion cannot be extended to monitoring of LR or normal deliveries. Before the use of universal intrapartum monitoring can be considered proven it is necessary to demonstrate 1) a significant improvement in perinatal outcome with monitoring, 2) an absence of the same degree of improvement with the selected monitoring approach, and 3) a favorable risk/benefit ratio in which the improvement in perinatal outcome outweighs the risk/benefit ratio associated with an extensive monitoring program. None of these important points have been clearly proved.

The present study failed to demonstrate any significant improvement as a result of a four-fold increase in monitoring, either in intrapartum or in early neonatal mortality rates. Not a single death can be attributed to the monitored or nonmonitored status. The results are in contrast with those of Amato (1) who found significantly fewer mortalities among monitored fetuses compared with nonmonitored fetuses both in LR and in LR deliveries.

Only a positive result in the form of a significant reduction of Apgar scores in the unselected monitoring program. The same result was obtained by Amato (1). We

consider the low morbidity in our material to be a result not only of increased monitoring but also of active management of labor of a short duration of labor due to active management and of intensive neonatal care (15, 16, 18).

It is not our intention here to discredit the concept that routine intrapartum monitoring represents an optimal method of surveillance of labor and access to continuous information. We simply wish to point out that the proof of better results in terms of perinatal mortality does not exist. Those who advocate the concept have it seems to us an obligation to criticize their program of health care throughout the prenatal to the neonatal care and to acknowledge that the optimal percentage of intrapartum monitoring depends on multiple variables. The appropriate percentage for each obstetric unit should be chosen according to its health care complex.

Apparently we are not alone in our feelings about the effects of staff versus the machine. Haverkamp *et al.* (7) compared the effectiveness of fetal monitoring with the effectiveness of auscultation of the fetal heart sounds and pointed out that the overall findings make it appear that it may be the staff, both nursing and medical, and the facilities that are important in achieving an optimal outcome rather than the use of electronic monitoring *per se*.

In the discussion following a presentation on fetal monitoring by Hon. Zannini and Quilligan (8) Goodlin stated that the staff and facilities may be the important feature adding. It seems most important that this question be resolved before every obstetric unit in the country feels obligated to spend thousands of dollars for fetal monitors in order to avoid lawsuits.

Since this statement little has been done to resolve the problem. We feel that further studies on the risk/benefit ratio in selective versus routine monitoring are mandatory.

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*Submitted for publication January 4 1979*

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## STUDIES IN NORMAL PREGNANCY

## III Fatty acid composition of serum phosphoglycerides and cholesterol esters

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Metabolic and hormonal influences on liver lipid metabolism are reflected in the relative fatty acid composition of lecithin. Thus a successive decrease in essential fatty acids (EFA) of the n 6 fatty acids during pregnancy would indicate an incipient EFA deficiency at the time of delivery. Furthermore reciprocal changes in linoleic and arachidonic acid during the 1st trimester with an increase in arachidonic acid could suggest an enhancement of the Greenberg Pathway (Pathway II) in lecithin synthesis presumably caused by estrogen influence. During the 2nd and 3rd trimester reciprocal changes in lecithin content of palmitic and stearic acid are similar to changes appearing under cholestasis. Furthermore suggested that variations in the utilization of linoleic acid in cholesterol esters (expressed as cholesterol 18:2/lecithin 18:2) might reflect changes in cholesterol esterification by the enzyme lecithin:cholesterol acyl transferase (LCAT). Then in late pregnancy a relation to a subclinical cholestatic liver engagement activity would increase as judged from this ratio as the increased linoleic acid in cholesterol esters.

Relative fatty acid composition of serum lecithin does not only reflect deficiency states of specific fatty acids (the essential fatty acids (EFA) of the n 6 fatty acids) but also hormonal and metabolic influences on the pathways of lecithin synthesis. The major pathway the Kennedy pathway (Pathway I) for lecithin synthesis preferentially results in the appearance of palmitic (16:0) acid in the 1 position and linoleic (18:2) or oleic (18:1) acid in the 2 position. Cholestatic conditions (8) as well as bile acids (9) appear to enhance this pathway. On the basis of the experiments in the human *in vitro* (19) as well as *in vivo* (2, 10, 11) reveal that estrogen enhances the Greenberg pathway (Pathway II) resulting in the appearance of more lecithin with stearic (18:0) acid in the 1 position and arachidonic (20:4) acid in the 2 position. Influences on one lecithin synthesis pathway appear to cause a reciprocal change in the

The aim of the present investigation was to study the metabolic and hormonal influences as they are reflected by the relative fatty acid composition of serum lecithin and cholesterol esters throughout normal pregnancy.

## MATERIAL AND METHODS

**Clinical material** Three different series of healthy pregnant women consecutively selected from the Maternal Welfare Unit: one series of non pregnant healthy women and one series of postpartum women consecutively selected from the department of Obstetrics and Gynecology were studied. **Series I (1st trimester)** Twenty pregnant women (mean age 24.4 range 14-40 years) with normal uncomplicated pregnancy were studied at 8-12 weeks (mean 10.6 weeks) of gestation.

**Series II (2nd trimester)** Twenty pregnant women (mean age 26.6 range 20-41 years) with normal uncomplicated pregnancy were studied at 20-24 weeks (mean 21.7 weeks) of gestation.

**Series III (3rd trimester)** Seventeen pregnant women (mean age 33.5 range 18-35 years) with normal uncomplicated pregnancy were studied at 31-37 weeks (mean 33.6 weeks) of gestation.

**Series IV (post partum)** Ten normal post pregnant women (mean age 24.2 range 20-28 years) were studied 2-5 days after an uncomplicated delivery following a normal pregnancy.

**Series V (non pregnant)** Eighteen non pregnant women (mean age 26.2 years range 19-34 years) with regular menstrual cycles and not using oral contraceptives were studied on the 1st or 2nd day of their menstrual bleeding and served as controls.

The women in the five series did not differ in mean age, had a normal body weight and the pregnant women a normal weight gain throughout pregnancy. Data in Series III, IV and V have partially been presented previously (8, 14, 15) and are only included for the purpose of comparison. Blood samples were drawn in the fasting state in the morning, centrifuged at 2 500 x g for ten minutes. The serum was then after immediately recovered, frozen and stored at -20 °C in glass tubes with teflon screw caps.

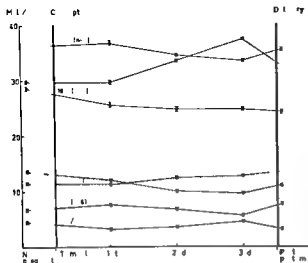


Fig 1 Relative composition of major fatty acids in serum lecithin at different stages of normal pregnancy in the non pregnant state and after delivery (post partum) 1st trimester = week 11 2nd trimester = week 22 3rd trimester = week 34 and post partum = 2-5 days after delivery Fatty acid data are given in mole per cent of methyl esters Mean  $\pm$  SEM

**Preparation of lipids and fatty acid methyl esters** Separation of lipids by thin layer chromatography (TLC) on Silica gel with the isolation of lecithin (developing medium chloroform methanol water 65/25/4 by vol) and of cholesterol esters was performed as described elsewhere (13). Preparation of fatty acid methyl esters was performed for lecithin according to the method of Olegård and Svennerholm (13) and for cholesterol esters according to Olegård (12).

Table 1 Relative composition of major fatty acids in serum lecithin at different stages of normal pregnancy Lecithin is given in mg/100 ml fatty acid data are given in mole per cent of methyl esters

Trimester	1st <sup>1)</sup>		2nd <sup>2)</sup>		3rd <sup>3)</sup>	
	Mean	SE	Mean	SE	Mean	SE
16:0	29.85	0.49	33.83	0.49	37.38	0.42
16:1 (n 7)	1.12	0.05	1.11	0.03	1.04	0.04
18:0	12.13	0.27	10.01	0.22	9.54	0.22
18:1 (n 9)	11.33	0.29	12.46	0.27	12.67	0.25
18:2 (n 6)	25.87	0.71	24.90	0.85	24.90	0.44
20:3 (n 6)	2.85	0.11	2.88	0.13	2.76	0.14
20:4 (n 6)	7.86	0.28	6.99	0.29	5.74	0.25
22:6 (n 3)	4.29	0.21	4.48	0.22	4.03	0.24
18:22 (n 6)	36.83	0.65	34.41	0.46	33.66	0.48
Lecithin	182.80	3.94	232.56	6.39	261.65	9.72
16:0/18:0	2.50	0.09	3.47	0.12	3.94	0.08
18:2/20:4	3.39	0.20	3.67	0.19	4.55	0.28

<sup>1)</sup> 1st trimester = week 11 of gestation (range 8-12) (n=20)

<sup>2)</sup> 2nd trimester = week 22 (range 0-24) (n=20)

<sup>3)</sup> 3rd trimester = week 34 (range 31-37) (n=20)

Table 2 Mean differences ( $\Delta$ ) in composition for fatty acids of serum lecithin at different stages of normal pregnancy 1st trimester vs 2nd trimester 1st trimester vs 3rd trimester Lecithin is given in mg/100 ml fatty acid data are given in mole per cent of methyl esters

	1st vs 2nd		1st vs 3rd
	$\Delta$	P	$\Delta$
16:0	+4.0	**	+7.5
16:1 (n=7)	0	-	-0.1
18:0	-2.1	**	-2.6
18:1 (n=9)	+1.1	**	+1.3
18:2 (n=6)	-1.0	-	-1.0
20:3 (n=6)	0	-	-0.1
20:4 (n=6)	-0.9	-	-2.1
22:6 (n=3)	+0.2	-	-0.3
18-22 (n=6)	-2.4	*	-3.2
Lecithin	+49.8	*	+79.0
16:0/18:0	+0.9	**	+1.5
18:2/20:4	+0.3	-	+1.2

0.05 level

0.01 level

0.001 level

**Gas liquid chromatography (GLC) of methyl esters** Tracts containing the fatty acid methyl esters were evaporated to a final volume of approximately 1  $\mu$ l and analyzed in a Perkin Elmer Model 4000 equipped with a flame ionization detector on a 200- $\mu$ m (3 mm in inner diameter) packed with 1,1-diethylene glycol succinate (DEGS) and Chromosorb W DMCS 80-100 mesh 100/100 as carrier gas and the column was operated at 150°C.

Individual esters were identified by a relative retention times of standards (Inc. L 209 and L 108) and phospholipid fatty esters of bull testis. Peaks were quantitated by the height by the width at half height. As shown by Svennerholm (18) impurities in solvents interfere with the determination of 20:5 (n 3) and 22:3 (n 9) and no data are given for these fatty acids.

In addition to the fatty acids presented in Table 1, 15:0 (n 3), 18:3 (n 3), 10:1 (n 9), 20:3 (n 9) and 22:5 (n 3) have been identified (18) but are not given because their concentrations were generally less than 0.1%.

**17:0 (heptadecanoic acid)** Perkin Elmer 1700 was used as internal standard. A known amount of 17:0 was added to the scraped off material of TLC spots before the gas chromatography procedure.

**Quantification of serum lecithin** Serum lecithin was quantified from the fatty acid content (obtained by GLC) using a nomogram. The equation  $Y = 1.66 X$  was used, where Y is the experimentally determined relationship (8) by gas chromatography and X is the amount of lecithin calculated from fatty acid content. For each chromatogram the fatty acid composition was calculated as percentage of area (weight per cent) of

**III Relative composition of major fatty acids in serum cholesterol esters at different stages of normal pregnancy** Fatty acid data are given in mole per cent of methyl esters fatty acids in mg/100 ml

ester	1st <sup>1)</sup>		2nd <sup>2)</sup>		3rd <sup>3)</sup>	
	Mean	S E	Mean	S E	Mean	S E
(n 7)	4.27	0.33	3.09	0.23	3.13	0.32
	12.89	0.24	13.18	0.27	12.16	0.23
(n-9)	2.87	0.19	3.77	0.27	3.7	0.26
	1.35	0.05	1.27	0.07	0.90	0.04
(n-6)	20.34	0.58	23.09	0.69	22.25	0.58
(n-6)	52.23	0.82	50.57	1.04	53.53	0.99
(n-6)	4.30	0.21	3.64	0.18	2.75	0.18
(n-3)	0.31	0.03	0.24	0.01	0.17	0.8
(n-6)	56.06	0.79	53.66	1.00	55.58	0.93
acids	58.15	2.51	73.11	3.43	90.16	3.59
0	9.78	0.36	10.87	0.61	13.57	0.62
20.4	12.85	0.80	14.48	0.76	20.8	1.6

trimester = week 11 of gestation (range 8-12) (n=20)

trimester = week 22 (range 20-24) (n=20)

trimester = week 34 (range 31-37) (n=20)

**esters** To obtain the mole per cent distribution conveniently used in metabolic discussions the area each peak in the chromatogram was estimated and divided by the corresponding molecular weight for each fatty acid methyl ester. This is described in detail elsewhere (8). **Statistical methods** Conventional methods were used for estimation of means, standard deviations and standard error of means. Student's *t* test was used to study differences between different groups and the dependent *t* test was used for analyzing differences between means within groups. Testing correlation coefficients between different variables due consideration was taken to the fact that some variables may have a functional relationship (i.e. serum cholesterol and its components). Values of  $p \leq 0.05$  were considered statistically significant (4).

## RESULTS

**Relative fatty acid composition of serum lecithin in 1st trimester** (Table I, Fig. 1) During the 1st trimester (week 11) (as compared to non pregnant) serum lecithin contained less stearic (18:0) ( $p < 0.01$ ) and linoleic (18:2) ( $p < 0.05$ ) acids while arachidonic (20:4) acid increased ( $p < 0.05$ ). The ratio of stearic and linoleic acid decreased ( $p < 0.01$ ). **Relative fatty acid composition of serum lecithin in 2nd and 3rd trimesters** (Table I and II, Fig. 1) In the 2nd (week 22) and 3rd (week 34) trimesters compared to the 1st serum lecithin fatty acid composition revealed certain characteristic changes. Linoleic (16:0) and stearic (18:0) acids were inversely related as 16:0 increased ( $p < 0.001$ ) and 18:0 de-

creased ( $p < 0.001$ ). Similarly oleic (18:1) acid increased ( $p < 0.01$ ) and sum of n-6 fatty acids showed a decrease with minimum values at week 34 ( $p < 0.001$ ). The ratio 18:2/20:4 increased in the 2nd and 3rd trimesters as compared to the 1st. Arachidonic (20:4) acid decreased in the 2nd ( $p < 0.05$ ) and 3rd ( $p < 0.001$ ) trimester.

**III Relative fatty acid composition of serum cholesterol esters in the 1st and 2nd trimester** (Tables III and IV) In the 1st trimester (week 11) arachidonic (20:4) acid ( $p < 0.05$ ) was more frequently utilized in the esterification of cholesterol while in the 2nd trimester (week 22) monoenoic acids i.e. 16:1 ( $p < 0.01$ ) and oleic (18:1) acid ( $p < 0.01$ ) were relatively more important.

**IV Relative fatty acid composition of serum cholesterol esters in the 3rd trimester** (Table III and IV) In the 3rd trimester (week 34) the composition of serum cholesterol ester fatty acid revealed (as compared to the 2nd trimester) an increased amount of linoleic (18:2) acid ( $p < 0.05$ ). The relative proportion of palmitic (16:0) to stearic (18:0) acids in cholesterol esters was lower in the 3rd as compared to the 2nd trimester ( $p < 0.01$ ) and ( $p < 0.001$ ) respectively. The ratio in cholesterol esters of 18:2/20:4 increased in the 3rd trimester ( $p < 0.001$ ) as did the ratio of cholesterol ester 18:2/lecithin 18:2.

Table IV Mean differences ( $\Delta$ ) in composition of major fatty acids of serum cholesterol esters at different stages of normal pregnancy 1st trimester vs 2nd trimester and 1st trimester vs 3rd trimester. Fatty acid data are given in mole per cent of methyl esters fatty acids in mg/100 ml

	1st vs 2nd		1st vs 3rd	
	$\Delta$	P	$\Delta$	P
14:0	-1.2		+0.1	-
16:0	+0.3	-	-1.0	-
16:1 (n-7)	+0.9	-	-0.1	-
18:0	-0.1	-	-0.4	-
18:1 (n-9)	+2.8	-	-0.8	-
18:2 (n-6)	-1.7	-	+3.0	*
20:4 (n-6)	-0.7	-	-0.9	-
22:6 (n-3)	-0.1	-	-0.1	-
18-22 (n-6)	-2.4	-	+1.9	-
Fatty acids	+15.0	-	+17.1	-
16:0/18:0	+1.1	-	+2.7	-
18:2/20:4	+1.6	-	+6.3	-

0.05 level    0.01 level    0.001 level



## DISCUSSION

In normal pregnancy there was no apparent reduction in essential fatty acids (EFA) (Fig. 1) during the 1st trimester but during the 2nd and 3rd trimester there was a successive decrease in all fatty acids of the *n* 6 series i.e. essential fatty acids. This might indicate an incipient EFA deficiency towards the end of normal pregnancy. Concomitant with the reduction in EFA there was an increase in monoenoic acids of the *n* 9 series in line with earlier experience in EFA deficiency (1, 6, 15).

Furthermore during the 1st trimester the relative fatty acid composition of serum lecithin revealed reciprocal changes in linoleic and arachidonic acids with a reduced ratio in 18:2/20:4. An increase in arachidonic acids in early pregnancy was previously described by Karkut *et al.* (9). We have found an increased relative content of arachidonic acid in serum lecithin after the administration of a natural estrogen e.g. 17 $\beta$  estradiol to oophorectomized women (17). We interpret the present data as the expression of an increased lecithin synthesis by Pathway II due to estrogen influence. Such an interpretation would be in line with the present knowledge of the hormonal conditions in pregnancy i.e. an increased estradiol excretion (7) and estradiol blood level (20).

In mid and late pregnancy i.e. in the 2nd and most pronounced in the 3rd trimester reciprocal changes in palmitic and stearic acids with an increased ratio in 16:0/18:0 and increased oleic acid would indicate an enhanced lecithin synthesis by Pathway I. A characteristic feature in cholestasis of pregnancy is the metabolic influence on lecithin synthesis by Pathway I (8). It is therefore tentatively concluded that during the 3rd trimester an enhanced Pathway I would even in normal pregnancy be an evidence for subclinical cholestatic changes in the liver.

Soon after delivery a reduction in this cholestatic influence on liver lecithin synthesis is suggested by a decrease in palmitic acid content ( $p < 0.001$ ) as well as an increase in stearic ( $p < 0.001$ ) and arachidonic acids ( $p < 0.001$ ) as well as an increase in stearic ( $p < 0.001$ ) and arachidonic acids ( $p < 0.001$ ) in serum lecithin (Fig. 1).

During the 3rd trimester a high utilization of linoleic acid in cholesterol esterification was shown. This was apparent from the ratio between CE 18:2/Lec 18:2 ( $p < 0.05$ ), i.e. linoleic acid content of cholesterol esters and lecithin as well as the increased linoleic acid content ( $p < 0.05$ ) in cholesterol esters. In view of the fact that linoleic acid is the preferable substrate in

cholesterol esterification by the enzyme lecithin:cholesterol acyl transferase (LCAT) (16) it is interesting to suggest that during the 3rd trimester an increased ratio CE 18:2/Lec 18:2 might be an expression of increased LCAT activity. This would also suggest that the moderate cholestasis during late pregnancy can enhance LCAT activity possibly through an increased enzyme protein synthesis in the liver as has been suggested in other forms of mild cholestasis (8).

## ACKNOWLEDGEMENTS

Mrs Margareta Medberg is acknowledged for technical assistance and Miss Annika Höfde for typing the manuscript. This project was supported by grants from the Swedish Medical Research Council (19C 2100).

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Submitted for publication July 27 1978

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# A PROTOCOL DESIGN FOR STUDYING ALTERATIONS OF PHARMACOKINETIC PARAMETERS DUE TO PREGNANCY

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**Abstract.** A study protocol is described by means of which useful and pertinent information on possible alterations of pharmacokinetic parameters due to pregnancy can be integrated. Whenever any drug is prescribed to a pregnant woman for medical reasons pharmacokinetic data for that drug can be obtained by determination of drug levels in plasma or serum or other possible tissues. The same data should later be obtained for an identical dose of the drug given to the same woman after pregnancy. By comparing pharmacokinetic parameters in the same woman before pregnancy and after — when she serves as her own pregnant control — clinically and statistically significant differences may be discovered with a comparatively small patient material. As in each case the drug that is prescribed is for medical reasons this protocol involves no undue risks for the pregnant woman or the fetus.

Pharmacokinetic studies of drugs are usually carried out in men rarely in women and very rarely in pregnant women. Due to physiological alterations in various organ systems during pregnancy the pharmacokinetics of most drugs is likely to change. This change may be of such magnitude that an adjustment in dosage or route of administration might be required. Various reasons why pharmacokinetic parameters may be altered during pregnancy as well as the reasons for further knowledge concerning such changes have been discussed by Ledger (1). Considering that treatment with various drugs during pregnancy occurs quite frequently it is surprising that so little is known about possible differences in absorption, distribution and elimination of such drugs in pregnancy as compared to nonpregnant women. The reasons for this is probably that studies of drug behavior in pregnant women often involve ethical problems. Animal studies will not provide pertinent data due to differences between species in dura-

tion of pregnancy in anatomy in histology and in physiology.

The protocol described below was used in an investigation of the pharmacokinetics of ampicillin in pregnant women (2). However the design of the study can easily be applied to other drugs prescribed to pregnant women. This study design provides sufficient data to calculate several pharmacokinetic parameters.

## MATERIAL AND METHODS

In this particular study on ampicillin pregnant women with urinary tract infections served as test subjects. All patients were otherwise healthy as judged by their histories, physical examinations and routine laboratory tests performed on blood and urine. All patients volunteered for the study which was approved by the Human Experimentation Committee of the Karolinska Institute.

The sequence of the study is shown in Fig. 1. Each pregnant woman was prescribed 10 days of treatment with oral ampicillin 0.5 g q.i.d. However the first dose of 0.5 g (Dose A) was given intravenously (i.v.) and comprised the first out of a total of four test doses (i.e. doses after which concentrations in plasma and urine were followed). The second test dose of 0.5 g (Dose B) was given orally not less than one week (washout period) after completion of therapy.

After delivery when the normal menstrual cycle had been reestablished and breast feeding had ceased the third test dose of ampicillin 0.5 g (Dose C) was given as a single i.v. injection. The fourth test dose (Dose D) was given as a single oral dose of 0.5 g not less than one week after Dose C. In this way each woman served as her own nonpregnant control.

Blood was sampled and urine was collected for determination of ampicillin levels. The sampling schedule is shown in Fig. 2.

Prior to administration of Doses B and D the patients had been fasting for at least 8 hours. Food and liquids were allowed 3 hours after dose administration. None of the patients were taking any other medication at the time of test doses.

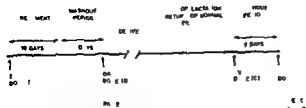


Fig 1 Sequence of test doses of ampicillin in relation to treatment delivery and breast feeding

The weight and gestational length of each patient were registered. Urine volumes were measured. Drug levels in plasma and urine were assayed according to routine methods.

The resulting values of ampicillin in plasma for each patient and dose were plotted against time on arithmetic as well as on semilog scale and provided a plasma concentration versus time curve. The arithmetic curve was used to calculate the area under the curve (AUC) which gave a rough measurement of the total amount of absorbed circulating and unexcreted drug and served as a basis for determination of the bioavailability of ampicillin. The semilog curve of plasma concentration versus time following i.v. administration to each patient was used for calculations of plasma half-life ( $T_{1/2}$ ), apparent volume of distribution ( $V_D$ ) and of plasma clearance ( $Cl_P$ ) of ampicillin.

Renal plasma clearance ( $Cl_R$ ) of ampicillin was calculated for each patient and dose based on mean plasma levels during each urine collection period and the amount of ampicillin recovered in urine during the same time.

Statistical analysis was performed with Student's *t* test for paired observations when these appeared to be normally distributed. For clearly skewed observations statistical analysis was performed by sign test for paired observations.

## DISCUSSION

Previous studies on serum levels of various drugs in pregnant women have usually been carried out simply as dose response studies where the response i.e. the serum levels resulting from a certain dose of a drug has been measured.

However it is a well known fact that pharmacokinetic parameters may vary vastly between individuals. Therefore serum levels or other pharmacokinetic data obtained for pregnant women would have to be strikingly different from such data found in a different population to be of statistical significance or the patient sampling would have to be very large.

In the above described protocol each woman who has been given a test dose of any drug when pregnant returns after pregnancy to be given additional test

doses thereby serving as her own nonpregnant control. This design allows statistical analysis to be based on paired observations. Thus a comparatively small patient sampling is required to detect alterations in pharmacokinetic parameters due to pregnancy. Provided test conditions are the same throughout the study.

Several pharmacokinetic parameters can be obtained if either the i.v. or the oral route of administration is used but by using both routes additional values can be obtained. By employing both routes above values can be obtained for peak levels,  $V_D$ ,  $T_{1/2}$ , bioavailability  $Cl_R$  and  $Cl_P$  which include metabolism. The dose per kg body weight can be calculated.

Any drug given sporadically or continuously for any length of time to pregnant women can be studied according to this protocol provided the sequence of the test dose in the course of treatment is the same during and after pregnancy i.e. single dose compared to single dose and morning dose is compared to morning dose in the case of continuous treatment.

The studying of pharmacokinetics of drugs during pregnancy is difficult for ethical reasons but this protocol is suggested for use only when there is a requirement for medical reasons. It does not imply any undue risks to the pregnant woman or to the fetus. The taking of several blood samples as well as the necessity of having each patient come back after delivery for additional test doses requires the test subjects to be well informed volunteers.

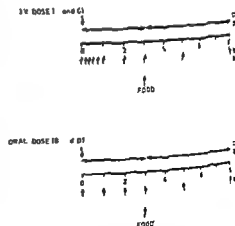


Fig 2 Sampling schedule for blood and urine and food intake at i.v. and oral test doses of ampicillin during the 8-hour observation period

his protocol proved itself to be a simple and use  
model. It can be utilized in full or with modifica-  
for all drugs prescribed during pregnancy the  
condition being that adequate laboratory  
methods exist to measure levels in urine, plasma or  
in other tissues of the drug to be studied. If  
for many and different drugs, much valuable in-  
formation about possible changes in pharmacokinetic  
parameters for drugs used during pregnancy could be  
gained. With such increased knowledge the dosages  
administered to pregnant women could be better and  
adequately calculated.

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*Submitted for publication December 12 1978*

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# STUDY OF AMPICILLIN LEVELS IN MATERNAL SERUM UMBILICAL CORD SERUM AND AMNIOTIC FLUID FOLLOWING ADMINISTRATION OF PIVAMPICILLIN

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## MATERIAL AND METHODS

Premature rupture of the membrane is often caused by intrauterine infections. Antibiotic prophylaxis is recommended but the effect has been debatable. Primiparous pregnant women received pivampicillin 350 mg 4 times daily. The ampicillin levels in amniotic fluid and in the serum of the fetus and of the mother were determined and related to the pivampicillin dose and time after administration. In the amniotic fluid the average concentration was 3.4 µg/ml which is considered therapeutic. In maternal and fetal serum the ampicillin concentrations were low and took a non-parallel course. To obtain the same ratio between ampicillin serum levels and MIC (minimum inhibitory concentration) in the pregnant as in the non-pregnant subject a dosage should be used.

The material consisted of 30 patients treated prophylactically with 1 tablet (350 mg) of pivampicillin 4 times daily. Samples were drawn from maternal blood, umbilical vein and amniotic fluid at parturition. The treatment period therefore varied from hours to several days.

Seventy-four per cent of the patients had premature escape of amniotic fluid; the others had planned section. In the latter group we had the opportunity of taking samples at a predetermined time after administration. All planned sections were, however, performed in the morning and we did not want to disturb the patients with drug ingestion during the night. In these series no amniocentesis was performed just for this purpose.

The ampicillin concentrations in the samples were measured by the disc diffusion method (BIODISK) *ad modum* Jalling (4). We used two standard series per set of samples and two discs per unknown sample. The values given represent the average of these.

## RESULTS

In order to obtain a basis for comparison the patients were grouped according to the number of hours after the last dose.

Table 1 The ampicillin level in maternal serum, umbilical cord serum and amniotic fluid in relation to the total dose administered and the time since the last dose (1-3 hours)

Patient no.	Total dose given (mg)	Time since last dose	Concentration level µg/ml		
			Maternal serum	Cord serum	Amniotic fluid
6	1 400	3 0	0	1 1	1 1
7	2 450	1 0	1 6	2 0	4 8
9	700	2 5	0 9	0 4	0 5
20	1 400	2 5	0 7	0 7	4 0
21	2 450	1 0	3 0	0 9	2 2
27	1 050	2 0	0 4	0	1 8
Mean			1 1	0 9	2 4

Premature rupture of the membrane occurs frequently in most maternity wards. Quite often a long time may pass between the escape and parturition. The risk of ascending infection is great and the delivery may be complicated (11). Prophylactic use of antibiotics is resorted to in this condition. The effect of such treatment is still a subject for discussion; no satisfactory answer has yet been given (3).

With the introduction of ampicillin various studies of its concentration in the fetal circulation have been carried out showing that both parenteral and oral administration produce therapeutic levels in amniotic fluid (3, 8, 12, 13, 14).

The purpose of the present study was to determine ampicillin levels obtainable in the amniotic fluid, the serum of the mother and the fetus in relation to time after administration.

Pivampicillin tablets (Pondocillin® LEO) were administered, this being the antibiotic most often used in our department and a similar trial with this had not previously been performed.



**Table II** The ampicillin level in maternal serum, umbilical cord serum and amniotic fluid in relation to the total dose administered and the time since the last dose (3 hours)

Pat no	Total dose given (mg)	Time since last dose	Concentration level $\mu\text{g/ml}$		
			Maternal serum	Cord serum	Amniotic fluid
11	1 050	3		0.2	3.2
12	1 050	3	0.4	0.5	2.3
13	1 400	3	0.8	0.7	5.6
14	1 400	3	1.1	1.2	4.5
15	1 750	3	1.2	1.4	5.5
16	1 750	3	2.7	1	6.4
17	1 400	3	0.7	0.7	3.1
Mean			1.0	0.8	4.4

The concentrations in maternal serum shown in Table I are lower than expected with an average of  $1.0 \mu\text{g/ml}$ . The specimens were taken shortly after administration of the drug.

The patients in Table II were given 350 mg 3–4 times the preceding day and 350 mg in the morning 3 hours before planned section. The values in the amniotic fluid are high with an average of  $3.4 \mu\text{g/ml}$ . The serum levels of mother and fetus are low.

The results in Table III vary more. Three single values in amniotic fluids are below  $1 \mu\text{g/ml}$ . The delivery took place after a long interval without medication.

As shown in Table IV there is mostly no measurable concentration in maternal serum 9–11

**Table III** The ampicillin level in maternal serum, umbilical cord serum and amniotic fluid in relation to the total dose administered and the time since the last dose (4–4½ hours)

Pat no	Total dose given (mg)	Time since last dose	Concentration level $\mu\text{g/ml}$		
			Maternal serum	Cord serum	Amniotic fluid
5	1 050	4	0.3	6.4	6.4
8	2 100	4½	0.4	1.3	3
17	1 050	4½	0	0.5	0.8
18	1 050	4	1.8	0.8	2.3
19	350	4	1.5	0.9	1.1
22	700	4½	1	0.8	0.5
28	700	4	0.0	1.5	2.6
29	1 050	4	1.2	1.5	5.4
34	700	4½	2.3	0.6	0.6
Mean			0.9	1.6	2.5

**Table IV** The ampicillin level in maternal serum, umbilical cord serum and amniotic fluid in relation to the total dose administered and the time since the last dose (5–12 hours)

Pat no	Total dose given (mg)	Time since last dose	Concentration level $\mu\text{g/ml}$		
			Maternal serum	Cord serum	Amniotic fluid
10	5 250	11½	0.0	0.8	4.1
23	700	12	0.0	0.3	0.3
24	700	11	0.0	1.1	4.1
25	700	11	0.0	0.8	5
26	700	11	0.0		4
31	1 050	9	0.7	3.5	2.2
32	1 050	5	3.2	1.5	3
33	1 750	7	4	3.1	1.1
Mean			1.0	1.4	4

hours after ingestion of pivampicillin but the concentration in amniotic fluid is still high.

The mean values summarized in Table V show the ampicillin concentrations in maternal serum, umbilical cords are low and take an almost per course while the concentrations in amniotic fluid reach higher levels and last longer.

The average concentration in the amniotic fluid of all these patients was  $3.4 \mu\text{g/ml}$  with means varying from 2.4 to  $4.2 \mu\text{g/ml}$  in the four courses. These values are assessed as being therapeutic. The highest value was  $8.6 \mu\text{g/ml}$ . Levels of 4–5  $\mu\text{g/ml}$  were found after 11 hours.

## DISCUSSION AND CONCLUSIONS

It is important to note that peak concentrations were reached in the amniotic fluid considerably later than in the maternal serum. This might be due to the fact that the greater part of the ampicillin in amniotic fluid

**Table V** Mean ampicillin levels in maternal serum, umbilical cord serum and amniotic fluid in patients after orally administered pivampicillin

	Time since last dose	Concentration level $\mu\text{g/ml}$		
		Maternal serum	Cord serum	Amniotic fluid
Table I	1–3 hours	1.1	0.9	2.1
Table II	3 hours	1.2	1.0	4.1
Table III	4–4½ hours	0.9	1.6	2.5
Table IV	5–12 hours	1.0	1.4	4.1
Mean		1.1	1.2	3.1

inates from the fetus = urinary secretion. Normal ampicillin reaches a high concentration in the = contrast when the fetus is dead the ampicillin es of amniotic fluid are low as shown by Bray has been assumed that the reason is cessation function

investigators have paid attention to the wal of amniotic fluid. Plentl showed that half the iotic fluid was renewed within 95 minutes (10) seems to indicate that ampicillin is transferred to mniotic fluid from the maternal circulation and rinary excretion of the fetus makes only a small ntribution. This study neither verifies nor weakens theories

ie ampicillin levels measured in the amniotic s indicate that prophylactic treatment with n in a dosage of 350 mg 4 times daily is uate

e concentrations in maternal serum are low com t with the usual distribution curve. These values spond well to the low ampicillin concentrations d in pregnant women in a study performed ily by Philipson (9) there the average concen n was 1 (2  $\mu\text{g/ml}$ ) one hour after ingestion of of pivampicillin. The author concludes that if ame ratio between ampicillin serum level and (minimum inhibitory concentration) is desired pregnant as in the non pregnant subject dou osage should be used

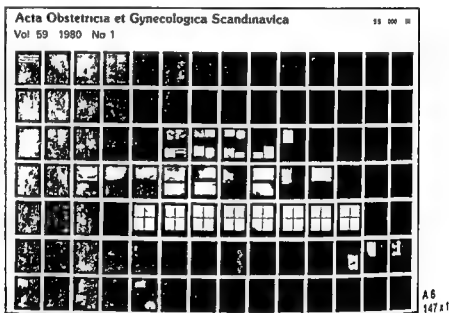
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Submitted for publication January 5 1979

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# EMOTIONAL STRESS IN CHILDBIRTH AND ITS MODIFICATION BY VARIATIONS IN OBSTETRIC MANAGEMENT

## Epidural analgesia and stress in labor

Peter C Buchan

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**Abstract** Emotional stress before, during and after labor was measured in 20 primigravidae by serial estimation of plasma 11 hydroxycorticosteroids and by stress assessment views. The anticipation of epidural analgesia and internal fetal monitoring was a significant source of emotional stress to an awaiting induction of labor despite explanation and repeated reassurance. During labor epidural analgesia reduced stress by abolishing so eliminating the progressive rise in 11 hydroxycorticosteroids normally seen throughout labor. Epidural analgesia does not, however, block the potential for the emotional response to stress and the physical work. Emotional stress and surgical trauma of delivery stimulate a considerable output of 11 hydroxycorticosteroids.

**Group II** Ten patients received epidural analgesia throughout labor. An epidural catheter was inserted and analgesia achieved using 0.2% per cent bupivacaine (without adrenaline) the first dose being administered as soon as the patient became aware of pain. All patients in this group had continuous intrauterine pressure recording and fetal heart rate monitoring throughout labor.

Five venous blood samples were taken from each patient: the first at 00.00 hours on the day prior to induction; the second at 08.00 hours on the morning of induction; the third when the patient was in established labor with the cervix at least five centimetres dilated; the fourth at the diagnosis of full dilatation; before the patient was allowed to bear down; and the fifth within one hour after delivery. Plasma 11 hydroxycorticosteroids were measured by the method of Mattingley (11).

## MATERIAL AND METHODS

Twenty primigravidae at between 40 and 42 weeks gestation were selected for induction of labor by amniotomy and by intravenous oxytocin infusion. No patient with hypertension, pre-eclampsia, adrenal or hepatic disease or a history of psychiatric illness or of corticosteroid therapy in pregnancy was included. Informed consent was obtained from all subjects following the antenatal stress assessment interview and after a full explanation of the study. By means of a semi-structured interview each patient had emotional stress assessed in the antenatal period during the first stage of labor and on the second postpartum day. All assessments were made by one interviewer who was not involved in the clinical management of the patient. The interviews the main areas of emotional stress investigated related to pain in labor, injury to or death of the patient, uncertainty about fetal well being and possible injury or death of the fetus. A score was allocated to each of the stress areas (three fetal and three maternal). Each was graded on a 0-10 scale giving a score out of sixty points. During the antenatal assessment and without knowledge of the results the patients were randomly allocated to one of two groups and informed of the intended protocol of their

## RESULTS

The stress scores for the antenatal (anticipation stress), intrapartum (experienced stress) and postpartum (retrospective stress) assessments are shown in Table I.

The anticipation stress was the same in both groups and there was no change in the experienced or retrospective stress compared with the anticipation stress in Group I. In Group II the experienced stress was significantly less than the anticipation stress ( $p < 0.05$ ) and the retrospective stress was also significantly less than the anticipation stress ( $p < 0.01$ ). The level of experienced and retrospective stress in Group II was significantly lower than in Group I ( $p < 0.01$ ).

The plasma 11 hydroxycorticosteroid levels in each stage in the two groups and the means and standard deviations are seen in Table II.

In Group I there was no difference between the antenatal and preinduction levels; the rise from the preinduction to the first stage of labor levels was significant ( $p < 0.001$ ) as was the rise from the first to the second stage of labor ( $p < 0.001$ ) and the rise from the second stage of labor to the postpartum level ( $p < 0.01$ ).

**Group I** Following induction of labor ten patients received 10 mg of pethidine intramuscularly for analgesia as frequently as it was requested and considered appropriate by the patient and medical staff.

Table I A Stress scores for the antenatal intrapartum and postpartum assessments in Group I patients

Pethidine analgesia	Patients									
	D W	M V	W L	J M	A M	B C	S M	M M	A A	C A
Antenatal assessment	48	26	37	52	18	20	54	54	8	11
Intrapartum assessment	56	36	24	50	8	24	24	40	12	12
Postpartum assessment	52	32	24	50	6	18	18	28	10	4

Table I B Stress scores for antenatal intrapartum and postpartum assessments in Group II patients

Epidural analgesia	Patients									
	J B	A C	L D	W B	L M	M C	V N	C K	S S	C F
Antenatal assessment	16	36	8	32	16	12	34	39	34	11
Intrapartum assessment	8	12	6	16	8	4	18	31	17	21
Postpartum assessment	6	9	4	12	6	4	10	23	18	11

In Group II there was a significant rise from the antenatal to preinduction levels ( $p < 0.02$ ). Although there was a slight fall from the preinduction to first and second stage of labor levels this change was not significant. The rise in 11 hydroxycorticosteroids from the second stage to postpartum levels was highly significant ( $p < 0.001$ ).

Comparing Groups I and II there was no significant difference between the antenatal preinduction and postpartum 11 hydroxycorticosteroid levels but both the first and second stage of labor levels in Group II were significantly lower than in Group I ( $p < 0.001$ ).

Patients who had an anticipation stress score greater than 30 had a significantly greater rise in their

11 hydroxycorticosteroids from the antenatal preinduction levels compared with those whose anticipation stress score was less than 30 ( $p < 0.05$ ). Patients with an experienced stress score greater than 30 had a significantly higher level of 11 hydroxycorticosteroids during the first and second stages of labor compared with those whose experienced stress score was less than 30 ( $p < 0.05$ ).

All patients in the study had vaginal deliveries. Two patients in Group I and three in Group II required assistance with forceps. There was no difference in their postnatal 11 hydroxycorticosteroid levels compared with patients having spontaneous deliveries.

Table II A Plasma 11 hydroxycorticosteroid levels for Group I patients (nmol/l)

Patient	Pethidine analgesia				
	Ante natal	Pre induction stage	First stage	Second stage	Post delivery
D W	1 173	1 501	1 595	2 448	2 095
M V	958	1 049	1 656	2 180	1 995
W L	831	1 018	1 248	1 775	2 360
J M	522	657	795	1 766	2 967
A M	822	1 010	1 813	1 951	2 103
B C	610	792	1 338	2 026	2 305
S M	729	742	1 455	2 255	2 857
M M	555	847	1 181	1 719	2 614
A A	599	712	1 449	2 092	3 331
C A	618	762	1 355	1 855	2 768
Mean	742	909	1 389	2 007	2 540
S D	196	237	269	227	417

Table II B Plasma 11 hydroxycorticosteroid levels for Group II patients (nmol/l)

Patient	Epidural analgesia				
	Ante natal	Pre induction stage	First stage	Second stage	Post delivery
J B	847	980	864	918	1 250
A C	1 054	1 394	1 005	1 206	1 100
L D	604	811	767	767	1 100
W B	845	1 024	1 032	1 063	1 100
L M	820	1 018	914	980	1 100
M C	897	1 145	980	1 071	1 100
U N	933	1 339	1 374	1 339	1 100
C K	1 195	1 540	1 339	1 071	1 100
S S	886	1 063	1 018	1 007	1 100
C F	723	847	834	811	1 100
Mean	880	1 116	1 013	1 015	1 100
S D	226	226	190	167	167

## DISCUSSION

According to Selye (13) stress is a non specific response of the body to any demand. Childbirth is both physical and emotional demands and the response may be measured by the rise in plasma hydrocortisone levels. Prior to the onset of labour most patients experienced some degree of anxiety often amounting to fear (12) and this was intensified prior to the induction of labor. This increased anxiety was reflected both in the stress score and in the plasma 11 hydrocortisone levels. Women who had no first hand knowledge of epidural analgesia or fetal monitoring viewed their intended delivery with some trepidation as shown by the rise in hydrocortisone levels between the antenatal and preinduction levels in Group II. This was despite the explanation and attempted reassurance concerning the procedure involved. Although patients in Group II had stated that they had had adequate explanation they nevertheless had greater stress as indicated by their increased corticosteroid levels in the period of labor.

At the onset of labor anticipation stress was augmented by pain and physical exertion which both stimulate adrenocortical activity (3-7). These factors produced progressively increasing adrenal stimulation which was not a maximal stimulus as shown by the gradual rise and by the response of the adrenal to dexamethasone given to patients in established labor by Campbell *et al* (3).

It is generally accepted that continuous epidural analgesia provides the most effective pain relief in labor (5). It does not directly affect the other stressors of labor but once pain is relieved anxiety and physical exertion are reduced. This was clearly seen in Group II by the fall in 11 hydrocortisone levels from the preinduction level in the first and second stage levels. This indicated that pain was the major stress factor in labor and when pain was relieved and the fetal condition monitored there was very little stress until the delivery. This is in agreement with the findings of Lederman *et al* (10) who showed a clear relationship between plasma cortisol and anxiety in labor and Burns (2) who showed that plasma cortisol was increased in patients with prolonged and painful labor.

The sharp rise in 11 hydrocortisone levels during delivery in Group II confirmed that despite adequate analgesia the knowledge that the baby was being born the physical exertion of bearing down and the surgical trauma of delivery and episiotomy

constituted an adequate stimulus to the hypothalamic-pituitary-adrenal axis. Epidural analgesia did not block the potential for the adrenal stress response it only blocked certain stress stimuli. This is of great importance as labor and delivery are times when surgical or haemorrhagic stress are common and the body's protective mechanisms must be able to respond when required.

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Submitted for publication March 13 1979

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1982

Date	Place	Name	Office
<b>1980</b>			
October 3-5	New Delhi India	3rd International Seminar on Maternal and Perinatal Mortality Pregnancy Termination and Sterilization	Hon. General Secretary The Fed. of Obstetric & Gynecological Societies of India Purandare Griha 31/6, Dr N. A. Purandare Marg, Bombay 400 007 India
Oct-Nov 23-3	San Marino Italy	The Gonadotropins: Basic Science and Clinical Aspects in Females	Leslie Nies Symposium Manager Soc. Symposia 11 Brooks Drive Boston MA 02184 USA
November 14-17	Madrid Spain	7 Congrès Européen de Médecine Périnatale	Professor de la Fuente Maternidad de la Ciudad Av. Generalísimo 177 Madrid 4
November 18-23	New Orleans LA USA	AAGL Ninth Annual Meeting Clinical Symposium on gynecologic endoscopy	American Association of Gynecologic Laparoscopists 11239 South Lakewood Boulevard Downey California 90241
November 20-22	Barcelona Spain	Symposium International Sobre Monitorización Prenatal	Instituto Dexeus Srtas. M. Llus Ana Baldrich c/Paseo de la Boqueria 67 Barcelona 17 Spain
November 22-25	Bombay India	First National Congress on Hormones and Human Reproduction	Mahendra N. Panikkar Organizing Secretary Dr. Jhaveri Hospital, Lady Hardinge Road Bombay 400 002 India
December 1-4	Kairo Egypt	Second Congress of the International Society for the Study of Hypertension in Pregnancy	Docent Hjördis Robbe Dept. of Obstet. and Gynecol. Karolinska Hospital S-104 01 Stockholm 60 Sweden
December 1-12	Melbourne Australia	Seventh UICC Training Course in Cancer Research	Dr. A. W. Burgess UICC Course The Walter and Eliza Hall Institute Royal Melbourne Hosp. P.O. Box 123 Victoria Australia

## EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMA INFECTIONS AMONG WOMEN IN NORWAY

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er to elucidate some of the epidemiological  
in the acquisition of *Toxoplasma* infections  
women were requested to answer a series of  
ir post natal periods. Of these women 27  
n infected with *T. gondii* 84 had old infec-  
ere non infected.

given indicated that frequent intake of raw  
ent travelling abroad especially to countries  
tion risks were of great significance for the  
among adults. Living in rural districts ap-  
sent a predisposing factor for the acquisition  
n. Contacts with animals especially catis  
n to increase the infection risk.

I reference to the results obtained a list of  
sero-negative pregnant women is proposed in  
nt congenital toxoplasmosis in the Scandina-

ity of infections with *Toxoplasma gondii*  
pendant on the prevailing climatic condi-  
and consequently shows great geographical  
tions (6). Other factors however which are  
or less related to the living habits of the popula-  
may also be of significance in the epidemiology  
man infections (7). Since the parasite may cause  
e disease in the fetus (for review see ref. 14) it is  
rally desirable to prevent the infection in the  
nant woman. It was therefore found of interest  
udy the possible role of some of these factors for  
acquisition of *Toxoplasma* infections among  
en of fertile age in Norway. The present study  
i with the results obtained from a questionnaire  
ered by a selected group of 196 mothers in the  
natal period. In approximately half of these  
en the sero reactions signalled old or recent in-  
ons with *T. gondii* the remaining women were  
negative and served as control subjects. The data  
ined would presumably provide information of  
rtance for the prevention of congenital tox-  
mosis in our country and perhaps also in the  
r Scandinavian countries.

### MATERIAL AND METHODS

**Study groups** The 196 women under study were all par-  
ticipants in a prospective investigation among 8 043 preg-  
nant women in the Oslo area based on serologic examina-  
tions (Dye test and indirect fluorescent antibody test (IgM-IFAT)) on paired antenatal blood samples collected on  
average in the 13th and the 35th gestational week (16).

Three groups of women were selected.  
*Group I* consisted of 85 women who had no detectable *Toxoplasma* antibodies in the two blood samples and were con-  
sequently considered to be non infected.

*Group II* comprised 84 women with low *Toxoplasma* an-  
tibody titers (10-100 IU/ml) and without specific IgM an-  
tibodies. In these women the acquisition had taken place  
months or years before the collection of the first antenatal  
sample and no change in their sero-reactions was observed  
during the current pregnancy.

*Group III* totalled 27 women who had been infected with *T. gondii* during or shortly before the current pregnancy. Dur-  
ing their gestational period 9 women showed sero-  
conversion (Dye test negative - 300 IU/ml). 2 showed  
substantial rise in titer ( $\geq$  eightfold rise in titers). 9 had  
high titers ( $\geq$  300 IU/ml) and 7 women had medium titers  
(100-300 IU/ml) and specific IgM antibodies.

The women in Groups I and II were randomly selected  
but care was taken to make the distribution in the groups as  
equal as possible with respect to age (mean age = 27 years).  
**Elements of study** Approximately 1-6 months after  
pregnancy the women were asked to complete a simple  
questionnaire in order to obtain information such as name,  
age, address, education, area (urban, suburban, rural) and  
type (apartment, house with garden, farm) of residence,  
contacts with birds and animals (cats, dogs, cows, sheep,  
etc.), eating and cooking habits (consumption of raw and  
cured meat, eggs, etc.) and visits abroad (name of country)  
in the past and especially during the current pregnancy.  
**Statistical method** The Chi square test with the Yates cor-  
rection factor was used for the calculation of significance.

### RESULTS

The information obtained from the questionnaire in  
the three groups and the statistically significant dif-  
ferences calculated are presented in Table 1. It will be



Table I Summary of the individual results

	Distribution (per cent)			Level of significance		
	I (n=85) uninfected	II (n=84) old infection	III (n=27) new infection	I/II	I/III	II/III
<b>Present situation</b>						
<i>Habitat</i>						
Urban district (apartment)	46	51	67			
Suburban district (house with garden)	52	48	33			
Rural district (farm)	2	1	0			
<i>Contact with animals</i>						
Cats	5	6	4			
Others	6	5	4			
Contact with birds	5	6	7			
<i>Eating habits</i>						
Frequent consumption of raw meat	11	13	33		$p \leq 0.01$	$0.001 < p \leq 0.05$
Frequent consumption of cured meat	30	33	31			
Frequent consumption of soft boiled eggs	15	25	27			
<i>Travel</i>						
Frequent travel abroad	8	19	31	$0.05 < p \leq 0.1$	$0.01 < p \leq 0.05$	
Frequent travel to risk countries	5	18	23	$0.05 < p \leq 0.1$	$p \leq 0.01$	
<b>Childhood</b>						
<i>Habitat</i>						
Urban district (apartment)	45	29	58	$0.05 < p \leq 0.1$		$0.01 < p \leq 0.05$
Suburban district (house with garden)	35	34	23			
Rural district (farm)	20	37	19	$0.05 < p \leq 0.1$		
<i>Contact with animals</i>						
Cats	44	50	48			
Others	40	57	41			
Contact with birds	25	31	26			
<b>Situation during pregnancy</b>						
<i>Travel</i>						
Travel abroad	30	39	52		$0.05 < p \leq 0.1$	
Change of residence	29	15	15	$0.05 < p \leq 0.1$		

seen that the distribution of the women with respect to location of present residence was practically identical in the different groups. The majority lived in urban or suburban districts; only three women were living on farms. A significantly higher number of the women with old infections (Group II) however had lived in rural districts during childhood compared to the non infected women (Group I). In keeping with this a significantly higher fraction among the non infected and recently infected (Group III) had lived in urban areas as a child compared to the women in Group II.

The groups were not found to differ in their replies about animal and bird contact. However the recently infected women (Group III) were consuming raw or under cooked meat more frequently than the women in the other groups whereas cured meat or soft boiled eggs appeared to be equally consumed in all three groups.

A significantly higher fraction among the infected women (Groups II and III) especially among those recently infected had visited foreign countries in particular those countries where the prevailing risk of *Toxoplasma* infections is considerably higher than in

our country. During the current pregnancy travel abroad was found to be most frequent among women in Group III whereas change of residence (i.e. domestic travel) was found to be more frequent among the women in Group I.

A statistical analysis was performed on some combinations of the answers given in order to demonstrate any positive or negative correlations. As shown in Table II travelling abroad was found to be strongly associated with frequent consumption of raw meat in both groups of infected women (Groups II and III). In contrast these habits were significantly less frequent among the women who had grown up on farms.

## DISCUSSION

The upper alimentary tract is believed to represent the main portal of entry of *Toxoplasma gondii* and the infection is acquired by contact with oocysts or by eating the tissue cyst in meat from infected animals (3, 8, 11). Hutchison and colleagues (9) were able to demonstrate the

Table II The correlation between some of the answers

	Total	Groups		
		I	II	III
<b>Positive correlation</b>				
Frequent consumption of raw meat — frequent travelling abroad	$p < 0.01$	not significant	$p < 0.01$	$p < 0.01$
<b>Positive correlation</b>				
Frequent consumption of raw meat — living on farm during childhood	$p < 0.01$	not significant	$0.05 < p \leq 0.01$	not significant
Frequent travelling abroad — living on farm during childhood	$p < 0.01$	$0.05 < p \leq 0.1$	not significant	not significant

oocyst in feces from cats orally infected with *T. du*. Since then special interest has focused on the cat as one of the main sources of human infections and recent investigations may suggest that this cat is as a permanent carrier of the infection (5). As shown in Table I the present study gave no evidence that intimate contact with cats or with other animals actually represented a risk of *Toxoplasma* infections in the women. This finding is in agreement with other investigations (1, 2, 20). However the oocyst shed from cats is remarkably resistant to disinfection in soil for several months (8, 15). The contamination risk might accordingly be expected to be higher among people handling soil and sand. This is supported by the fact that the prevalence of *Toxoplasma* antibodies in Norway (18) as well as in other countries (13) has been found to be higher among people living in rural as opposed to urban areas. Among the women under study a significantly higher fraction of those with old infections (Group II) had grown up in rural areas compared to the women in the other two groups whereas the location of the residence as such appeared to be of no importance in either of the groups. This again might indicate that people living in rural districts are more exposed to contamination from infected soil during their childhood than those living in urban areas. As pointed out by several investigators eating raw meat represents perhaps the main source of human infections (2, 12). Meat from domestic animals (e.g. pigs and cattle) and wild animals (e.g. hares and deer) in Norway has been shown to contain oocysts (21). However the traditional Norwegian eating habits favor well-done meat which usually has been stored frozen both handlings effectively destroying the infective tissue cysts (8, 22). In recent years the consumption of raw or semi raw meat (roast

beef, beef tartar etc.) has become more common. As shown in Table I raw meat was consumed significantly more often among the infected women especially among those recently infected compared to the non infected women. Moreover raw meat was found to represent the only dietary item which seemed to entail an increased risk of infection. Frequent intake of soft boiled eggs for instance (10) did not correlate with an increased risk of *Toxoplasma* infection as also found by Berger and Piekarski (1).

Special attention should be drawn to the fact that travelling abroad was more common among the infected women in particular among the recently infected ones ( $p < 0.01$ ). Scandinavians will naturally be exposed to an increased risk of *Toxoplasma* infections when visiting South European countries. Desmonts and Couvreur (4) have reported that cases of congenital toxoplasmosis in Paris are relatively frequent among Scandinavian women who marry in France. In the prospective serologic study in the Oslo area 13 out of 8 043 women were found to have become infected during pregnancy of whom two women most probably had been contaminated during a visit to Southern Europe (16). Since the frequency of *Toxoplasma* infections in our country may vary from 2 per cent in the North of Norway to about 30 per cent in the South (prevalences at 20 years of age) (18) travelling within our country may presumably also lead to an increased infection risk. This could not be confirmed in the present study (Table I).

As shown in Table II international travelling was found to be strongly associated with a frequent intake of raw meat in the groups of infected women (Groups II and III) while these habits were significantly less common among the women who had grown up on farms. It is therefore tempting to suggest that socioeconomic factors might be of some importance for the epidemiology of *Toxoplasma* infections in our

country. The fact that the prevalence of *Toxoplasma* antibodies in the Oslo area has been found to be higher in the wealthier districts of the city (17) constitutes further evidence for this theory. It is conceivable that urban citizens having a higher income visit foreign countries more often and are more apt to adopt foreign dishes in their cooking such as raw meat. At the same time people who have lived on farms during their childhood may be protected from *Toxoplasma* infections through their living habits as adults.

The prevention and control of congenital toxoplasmosis are no doubt accomplished most effectively by means of routine serologic screening of pregnant women since this may lead to rapid treatment of the infected children. Nevertheless prophylactic measures may be of great importance in this connection and women should generally be advised to take certain special precautions during the gestational period (7, 15, 19). Contact with cats should preferably be avoided, disposable gloves should be worn when handling cat feces or gardening, the hands should be thoroughly washed following contacts with raw meat, sand and soil as well as before eating. Consumption of raw meat should be abandoned. As pointed out in the present investigation, women from Norway and other Scandinavian countries should be especially advised to avoid travelling abroad in particular to high risk countries during a pregnancy.

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Submitted for publication June 6 1980

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## COMBINED ESTROGEN AND PROGESTOGEN FOR THE MENOPAUSE

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**Abstract** A single blind pilot study using combined estrogen and progestogen therapy was undertaken in 25 patients over a period of six months in an attempt to evaluate symptomatic control, the acceptability or otherwise of bleeding patterns and the effect on endometrial pathology. Three different progestogens were employed, i.e. norgestrel, norethisterone acetate and each was given in combination with estradiol valerate in a cyclical and a continuous regime.

Symptomatic control was good in all groups but there was a mild recrudescence of some symptoms during the free interval in the cyclical groups. Regular bleeding was reported in almost all cases receiving cyclical therapy and unscheduled bleeding was noted in only one continuous regime.

Endometrial hyperplasia did not occur following treatment in any of the groups and there was a return to the normal histology in two patients in whom hyperplasia existed prior to treatment. These results support the view that endometrial hyperplasia is least likely to occur if progestogens are administered in combination with estrogens. There were no obvious differences between the effects of the two progestogens.

The relationship of postmenstrual unopposed estrogen therapy and endometrial carcinoma has been disputed (4, 5, 9, 10, 13) however a dose response association has been demonstrated between this type of therapy and endometrial hyperplasia (2, 12). Few of the possible uses of long term estrogen therapy to prevent the occurrence of an osteoporotic fracture (1, 6, 7, 8) there has been obvious concern over risks involved.

Recently data have been published showing that the addition of a progestogen may reduce the incidence of endometrial hyperplasia (2, 6, 12) particularly in the work by Sturdee *et al.* (11) where the incidence of hyperplasia was also shown to be related to the duration of progestogen therapy being 12 per cent in cyclical unopposed estrogen therapy, 8 per cent in cyclical estrogen therapy with a progestogen and 1 per cent during the last 5 days of the treatment cycle.

and nil when the progestogen was added during the last 10-13 days.

Preparations are available with a progestogen added in the latter half of the cycle but in view of the reported increased incidence of endometrial carcinoma with the use of sequential oral contraceptives (3) and lack of any such correlation with the combined oral contraceptive it was decided that a pilot study of cyclical combined estrogen and progestogen preparations should be carried out in menopausal women. Two different progestogens were used in order to study their relative effects.

Some of the patients were given continuous therapy to determine whether the effects of these combined preparations could be extended to provide continuous symptomatic relief and freedom from bleeding without overstimulating the endometrium. One group received the estrogen in a twice-daily dose to see if there was better symptom control.

## MATERIAL AND METHODS

Patients with climacteric symptoms who were at least 6 months postmenopausal were randomly allocated to 5 different treatment regimes with 5 patients in each group. The duration of treatment was 11 cycles.

- A Cyclical estradiol valerate 2 mg + norgestrel 500 µg daily 21 days out of 28
- B Continuous estradiol valerate 2 mg + norgestrel 500 µg daily
- C Continuous estradiol valerate 1 mg b.i.d. + levonorgestrel 250 µg daily
- D Cyclical estradiol valerate 2 mg + norethisterone acetate 1 mg daily 21 days out of 28
- E Continuous estradiol valerate 2 mg + norethisterone acetate 1 mg daily

A detailed assessment of menopausal symptoms and signs was made before treatment and at 1, 3 and 6 months later.

The occurrence of withdrawal bleeding and unscheduled bleeding was recorded on diary cards.

An out-patient curettage was undertaken before and after the 6 months treatment.

Table I Frequency of hot flushes

		Treatment groups				
	Months	A	B	C	D	E
Hot flushes†	0	5	5	3	2	3
	1	3 (3)	3	3	2 (1)	1
	3	1 (4)	3	3	2 (1)	1
	6	1 (4)	2	1	1 (1)	0

one drop-out two drop-outs † no. of women with complaints  
Numbers in parenthesis are for the 7-day treatment free period

## RESULTS

**Symptomatology** Eight presenting symptoms were of interest the commonest being hot flushes which occurred in 78 per cent of patients followed by depression in 61 per cent night sweating 39 per cent insomnia 35 per cent day sweating 30 per cent dry vagina 26 per cent frequency of micturition 26 per cent and dyspareunia 22 per cent

With the numbers used it is not possible to see if any of the changes over time with treatment are statistically significant. However in addition to the reduction in the number of patients having the various symptoms almost all manifested a reduction in the frequency and intensity of symptoms. There was a mild clinically observed recrudescence of such symptoms as hot flushes and sweating during the tablet free interval in the cyclical groups. An example of these changes can be seen in Table I which shows the number of patients complaining of hot flushes during the study.

There were no apparent differences between those given estrogen once as opposed to twice daily nor between the two different progestogen regimes.

**Bleeding** In the cyclically treated groups there was no unscheduled bleeding recorded. All of the group A patients reported withdrawal bleeding of slight ( $n=4$ ) or normal ( $n=1$ ) intensity. Of the 27 cycles recorded no blood loss was reported in 2. In group D one patient had no diary card and one was lost to follow

up of the 18 cycles recorded no bleeding was recorded in 4. Intensity was recorded as slight or none.

In the continuously treated groups B, C and D intermittent spotting was recorded by all but 2 patients and was annoying in some. One patient respotting or bleeding on 48 days before withdrawal after 3 months therapy.

**Endometrium** The incidence and histology of curettings obtained before and after treatment are shown in Table II. The main changes shown are an increase in the number of curettings obtained from 7 per cent before treatment to 33 per cent after treatment and the conversion of a proliferative endometrium in the pretreatment sample to secretory endometrium after treatment. All 7 patients having a proliferative endometrium in the pretreatment sample inhibited this change. Of the 14 patients who had curettings before treatment 7 had no curettings in review 6 had a normal endometrium (1 proliferative 5 secretory) and 1 withdrew.

Two patients who had hyperplasia before treatment were reported to have a normal endometrium 10 months treatment (1 proliferative and 1 secretory). The pretreatment results were not available at commencement of treatment and it was considered reasonable to continue in both cases on the basis of the presence of a progestogen.

There were no obvious differences which correlated to the different progestogens.

**Side Effects** One patient in the cyclical group reported the withdrawal bleeding and also complained of weight gain. In the continuously treated group spotting was the commonest volunteered complaint followed by breast tenderness in 4 patients, weight gain in 2 and nausea in 1.

## DISCUSSION

The relationship of the estrogen dose and the incidence of a progestogen to the incidence of endometrial hyperplasia or atypical hyperplasia of the

Table II Incidence and histology of curettings

	No. of patients having curettage	Curettings obtained	Histology of Endometrium		Hyperplasia
			Proliferative	Secretory	
Before treatment	23	9 (39)	7	—	2
After treatment	22	15 (68)	2	13	—

the figures within parentheses denote per cent

um was demonstrated by Campbell *et al* (2) y showed an incidence of either form of endomet hyperplasia of 35 per cent in the cyclical unopposed high-dose estrogen group with a reduction to per cent in the unopposed low dose estrogen ap a further reduction to 8 per cent in the cyclical 1-dose estrogen group when a progestogen was ad for 7–10 days and an absence of hyperplasia in cal low dose estrogen group with a progesto- added for a similar period. Similar figures by head *et al* (12) support this finding.

figures quoted earlier by Sturdee *et al* (11) v a similar picture except that in the case of con us estrogen administered by means of an im together with 5 days of progestogen orally giv edically to induce monthly withdrawal bleeding incidence of hyperplasia was 28 per cent and there also one case of endometrial carcinoma. It was until the duration of progestogen treatment was ided to 10–13 days that the incidence of hyper fell to zero.

in this study the failure to demonstrate hyper in any of the treatment groups and the disap- mce of hyperplasia in two instances support the sion that endometrial hyperplasia is less likely associated with a combined estrogen and pro- gen preparation or when the progestogen is pre d for at least 10 days during each cycle.

irregular spotting in the continuously treated renders this particular combination unaccep- clinically and although there is the inconve of regular withdrawal bleeding and a recrudesc of symptoms during the tablet free interval in chical groups this would appear to be a neces accompaniment to the safe use of post meno- hormone therapy.

is not yet been ascertained however whether a ned estrogen and progestogen regime has dis- dvantages over the sequential preparations with ffs of a progestogen added or what the optimum schedules are for the various constituents.

#### ACKNOWLEDGEMENT

anks to Dr K D MacRae Charing Cross Hospital help in the analysis of the data.

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Submitted for publication November 21 1978

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1982

Date	Place	Name	Office
<b>1981</b>			
January 26-31	Mexico City Mexico	Pan American Congress of Andrology	Gerald Bagatzinski Congr Adm 31600 West Chicago Livonia, MI 48150 USA
March 14-18	Atlanta USA	37th Annual Meeting of the American Fertility Society	The American Fertility Society 1608 13th Avenue South Suite 101 Birmingham Alabama 35205 USA
March 22-26	Berlin West Germany	11th World Congress of Human Reproduction	Dozent L. Meitler Frauenkln. d. L. Hegewischstr 4 D 2300 Kiel
March 27-28	Modena Italy	Symposium on Recent Advances on Patho- physiology of Amniotic Fluid	Scientific Secretariat Dr G. C. Di Istituto di Clinica Ostetrica e Gine- cologica Policlinico Via del Pozzo 41100 Modena Italy
April 15-17	Gorizia Italy	International Course on Ultrasound in Obstetrics	Filippo Destro Organizing Secretary Dept. of Obstetrics and Gynecology City Hospital Via Vitorio Veneto 1 34170 Gorizia Italy
May 17-24	Dubrovnik Yugoslavia	4th European Congress on Ultrasonics in Medicine	Professor Asim Kurjak Ljubljana Stube 41000 Zagreb Yugoslavia
June 9-12	Ostend Belgium	Third International Congress on the Menopause	The International Menopause Socie- ty 8 av Don Bosco 1150 Brussels, Belgium
July 1-4	Graz Austria	First International Symposium on Minimal Invasive Cancer (Microcarcinoma)	Secretariat First Symposium on Minimal Invasive Cancer P.O. Box A-1014 Wien Austria
July 24-28	Cambridge England	XIII Acta Endocrinologica Congress	Conference Services Ltd XIII Acta Endocrinologica Congress 38 Grosvenor London SW7 3EY England
Sept Oct	Athens Greece	Vth European Congress on Sterility (ESCO)	Secretariat Prof Dr A. Senn Klinik der Universität Kiel Hirsch- strasse 4 2300 Kiel 1 West Germany
October 25-31	Melbourne Australia	Eight Asian Congress of Obstetrics and Gynecology	The Organizing Secretary VII Congress of Obstetrics and G G.P.O. Box 2195T Melbourne VIC Victoria Australia
<b>1982</b>			
October 17-22	San Francisco CA USA	Xth World Congress of Gynecology and Obstetrics	Xth World Congress of Gynecology Obstetrics c/o The American Coll Obstetricians and Gynecologists One East Wacker Drive Suite 1000 Chicago Illinois 60601 USA

# URETHRAL PRESSURE PROFILE IN CONTINENT WOMEN FROM CHILDHOOD TO OLD AGE

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**Abstract** One hundred and sixty nine urinary continent women were examined with simultaneous urethro-metry using a dual microtip-catheter. It was shown that the maximum urethral pressure and the urethral length varied from infancy to the age of 20-25 years. After the values of these parameters decreased with increasing age. The bladder pressure remained constant in different age groups.

In six women aged between 20 and 25 years the urethral pressures were measured three times during a menstrual cycle. No correlation between the fluctuating estrogens, genital catecholamines and the urethral pressure or the urethral length was found.

In the last two decades methods have been evolved for determining the urethra's intraluminal pressure which is important for urinary continence (1-7). The difference between maximum urethral pressure (MUP) and bladder pressure (BP) is called the closure pressure (CP). When CP is positive the individual is continent; when it is zero or negative she is incontinent.

The main methods have been employed for the measurement of urethral pressure: 1) open end filled balloon catheters (8); 2) open end catheters with constant flow (6, 11); 3) microtransducers placed intraluminally in the urethra (3, 4, 5).

Although the first two methods have been used extensively in the past, the equipment is difficult to handle and calibrate. Furthermore, with open-end catheters with constant flow, problems arise in measuring the urethral closure pressure, which is important when investigating urinary incontinence. Both methods may give a great variety of ar-

bitrary pressure values. The pressure can be measured simultaneously at rest, under stress conditions, and during filling of the bladder.

Each method has its own normal value. With open end catheters the pressure comprises both the intraluminal pressure and the resistance to flow. With balloon catheters the measuring section is usually so large that the recording represents the sum of different pressures over a rather long distance. This impedes calculations of the true intraluminal pressure. The microtransducer measures just the intraluminal pressure in an extremely small area (approximately 0.75 mm<sup>2</sup>). The aim of the present investigation was to determine UP, BP and CP — as measured by microtransducer catheters — in healthy women of different ages. Furthermore, these parameters were studied during the menstrual cycle, since it has been claimed that they may fluctuate with estrogen and gestagen levels (10).

## MATERIAL AND METHODS

The study comprised 169 healthy women. They were divided by age into thirteen groups: 6-15 years, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70 and >71. The youngest girls had been examined because of symptoms indicating urinary incontinence; those with no objective signs of incontinence (see below) were included in the present study.

The middle age groups consisted mainly of healthy volunteers with no urological symptoms. Some women had been admitted to the department because of carcinoma in situ of the cervix. Bacterial cultures were negative in all patients and cystoscopy was normal. The subjects were examined with simultaneous urethrocystometry including measurements of the urethral pressure profile according to the Asmussen-Ulmsten technique (3, 4, 5). The bladder was filled at a rate of 1 ml/min with saline heated to body temperature. Three consecutive urethral pressure profiles (UPP) were registered at rest at a bladder volume of 200 ml. All patients were examined in a semi-seated position.

Microtransducers measure the pressure in a small area with high accuracy and reproducibility. A dual catheter, bladder pressure and urethral



Table 1 MUP, BP and CP in different age groups

	Age groups											
	6-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70
Parity	0	0.05	0.04	0.83	1.90	2.11	1.78	1.75	1.81	1.57	1.3	1.30
MUP	97±6	106±5	110±4	94±5	104±8	81±7	89±5	80±8	84±6	82±7	68±5	66±9
BP	70±2	21±1	20±1	21±1	0±2	16±2	22±1	17±2	14±1	21±2	22±3	17±1
CP	77±7	85±4	90±4	73±5	84±8	65±7	67±5	63±7	60±6	61±7	46±7	49±9
No	12	18	24	18	11	9	18	12	15	14	7	6

Mean ± SE

Urinary continence was recognized in three ways

- 1 A negative history of urinary stress incontinence
- 2 No observed leakage of saline from the outer urethral meatus
- 3 A positive urethral closure pressure both at rest and at stress

Six nulliparous women, mean age 22 years (20-25) were investigated in the same manner at various phases of the menstrual cycle, i.e. early at midcycle and late. On these occasions blood samples were taken to determine total estrogens, progesterone and the catecholamines, dopamine

adrenaline and nor adrenaline. The technique for these determinations have been described elsewhere (10).

**Calculated parameters** The mean of three consecutive UPPs at a bladder volume of 200 ml was determined and used to calculate MUP, BP, CP, FUL and AUL (Fig. 1).

**Statistics** The variations in MUP and BP in different groups were tested for significance by means of analysis of variance (F test) (1).

Urethral length (FUL and AUL) was analysed by Student's t test.

## RESULTS

**Urethral pressure** As seen from Table 1 and Fig. 1 MUP increased from a mean of 97 cm H<sub>2</sub>O (59-115) in the youngest age group to 110 cm (range 76-154) in the age group 21-25 years. Increase was not statistically significant. After the

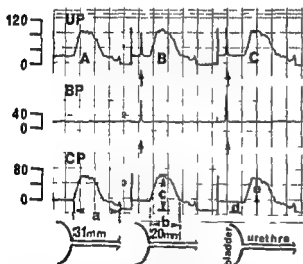


Fig. 1 A, B and C represent three consecutive urethral pressure profiles in a female. The arrows indicate the presence of both transducers in the bladder as the difference between UP and BP at that point is zero.

UP = Urethral Pressure

BP = Bladder Pressure

CP = Closure Pressure

a = absolute urethral length

b = functional urethral length

c = maximum urethral pressure

d = bladder pressure

e = closure pressure

Paper speed = 2.5 mm/sec

Catheter withdrawal speed = 2.5 mm/sec

Pressures in cm H<sub>2</sub>O

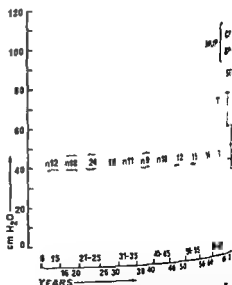


Fig. 2 Maximum Urethral Pressure (MUP), Bladder Pressure (BP) and Closure Pressure (CP) in females in different age groups. SEM = Standard Error of the Mean.

## I Urethral lengths in different age groups

Age groups												
6-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	≥71
27±2	27±1	30±1	30±2	29±2	27±2	27±1	31±2	28±1	29±1	25±2	23±2	26±2
31±2	34±1	37±1	37±2	36±2	32±2	35±1	37±2	35±1	35±1	32±3	30±2	33±2
13	13	24	13	11	9	13	12	15	14	7	4	7

SE

years MUP decreased with increasing age. For the difference between the age group 21-25 and women over 36 years was significant ( $p < 0.05$ ). No definite decrease was found in relation to age.

**Urethral pressure** The bladder pressure did not differ significantly between the age groups; it remained constant at 20 cm H<sub>2</sub>O (at a bladder volume of 100 ml).

**Urethral pressure** Since BP was more or less constant, urethral pressure showed the same significant increase with increasing age as MUP (Table I and

**Urethral length** FUL increased from a mean of 27 mm (range 18-41) in the youngest age group to a maximum of 31 mm (range 23-43) in the age group 46-50 years; it decreased thereafter to a mean of 25 mm (range 18-36) at 70 years and older (Table II). These differences were significant ( $p$

$< 0.05$ ). The difference in FUL between women before and after the menopause was significant ( $p < 0.05$ ).

**Absolute urethral length** AUL showed the same tendency as FUL and the same statistical significance for shortening after the menopause. However, in all age groups AUL exceeded FUL by about 5-10 mm. **MUP, BP, CP, FUL, AUL and catecholamines during the menstrual cycle** No definite correlation was found between continence maintaining parameters (MUP, BP, CP, FUL, AUL) and estrogen and gestagen levels during the menstrual cycle (Table III).

The catecholamines (dopamine, adrenaline and noradrenaline) showed very small variations during the menstrual cycle and could not be responsible for the variations in MUP during a series of measurements in the individual woman. Nor did they influence urethral length (Table III). Only one woman had a detectable level of dopamine.

## III Dependence of estrogens, gestagens and catecholamines on the urethral pressure profile in sixty nulliparae

Age	Day <sup>1</sup>	MUP <sup>2</sup>	BP <sup>2</sup>	CP <sup>2</sup>	FUL <sup>3</sup>	AUL <sup>3</sup>	Estrogen <sup>4</sup>	Progesterone <sup>4</sup>	Adrenaline <sup>4</sup>	Noradrenaline <sup>4</sup>	Dopamine <sup>4</sup>
22	7	135	24	111	27	36	0.30	1.7	0.1	1.1	-
	14	138	24	114	25	35	0.70	2.0	0.6	1.4	-
	22	141	24	117	27	37	0.61	14.1	0.6	1.0	-
25	9	88	20	88	34	42	0.31	2.2	0.3	1.2	-
	12	79	18	63	31	44	1.24	1.6	0.4	1.7	-
	23	84	20	84	32	43	0.93	9.9	0.3	1.8	-
28	17	125	28	97	30	42	0.98	2.3	0.3	0.8	-
	24	128	28	100	28	37	0.68	3.0	0.6	1.2	-
	29	116	28	83	27	36	0.90	24.9	0.6	1.0	-
22	6	110	24	86	26	30	0.46	1.5	0.95	1.8	0.16
	12	124	24	100	26	28	0.76	2.0	1.16	1.7	0.6
	22	109	24	85	23	28	1.11	37.3	1.25	1.1	0.4
20	8	134	28	106	29	34	0.18	1.3	1.3	-	-
	12	125	24	101	30	34	0.21	2.4	1.2	2.9	-
	22	135	24	111	31	40	0.43	19.5	1.2	2.2	-
21	9	120	20	100	32	36	0.26	1.1	0.7	1.4	-
	13	137	16	121	29	35	0.40	<1.0	0.7	1.1	-
	23	133	28	105	29	34	0.32	<1.0	0.7	1.7	-

<sup>1</sup>cycle day, <sup>2</sup>cm H<sub>2</sub>O, <sup>3</sup>mm, <sup>4</sup>pmol/L.

## DISCUSSION

The decrease in urethral pressure with increasing age was demonstrated in 1961 by Enhörning (9). It has been claimed that MUP is highest in childhood (7, 12). This study however shows an increase from infancy to adolescence though the results do not provide any explanation for this. The increase does not seem to be related to puberty. If puberty (with an increasing production of estrogens) and the climacterium (with decreasing estrogen levels) did influence urethral pressure, a marked increase would be expected around puberty and a marked decrease after the age of 50. Moreover, one would expect a correlation between urethral pressure and estrogen levels during the menstrual cycle. The present study showed no such correlation and the decrease in urethral pressure with increasing age was continuous. Furthermore, the estrogen level in ovulating women is much the same at 21–25 and 40–45 years of age (2, 17).

In an experimental study in the human female we found that blood pressure was responsible for 1/3rd of the maximum urethral pressure (15). The individual tracings revealed a more marked periurethral arterial pulsation in the youngest age groups compared with the older. The urethral pressure decrease is therefore more likely to reflect a loss of tonicity in the urethral wall and thicker vessel walls with increasing age thereby diminishing pressure transmission to the urethral lumen.

Another study has shown that high doses of estrogens in postmenopausal women increase the maximum urethral pressure only slightly. Nor did the gestagen levels seem to influence MUP in these women (16).

In the present investigation the six women examined during the menstrual cycle showed no correlation between estrogen levels and MUP. Neither was there any correlation between serum levels of the catecholamines dopamine, adrenaline nor adrenaline and MUP.

Parity might be one of the causes of the decrease in urethral pressure with increasing age. This could theoretically explain the onset of the decrease from 25 years of age. However, although the material is too small to be divided into parous and non parous, the individual pressure tracings showed that MUP fell with increasing age even in the non parous women.

The length of the urethra is held to be of great importance for continence (13). It is therefore of interest to find that urethral lengths do shorten after the menopause. In another study I found that high

dose estrogen treatment elicited a lengthening of the urethra but this lengthening did not correlate significantly with improvements in urinary continence (16).

At physiological levels however it may be concluded that estrogens and gestagens as well as catecholamines do not influence MUP or urethral length. This agrees with Ek *et al.* who found an increase in MUP after therapeutic doses of estrogen in stress incontinent women (8).

According to Edwards and Malvern (7) MUP in healthy women measured by open-end catheter at constant flow can be calculated from the formula:  $MUP = 1.5 \times \text{age} + 10$ .

Pressures measured with the microtip catheter were however higher as a rule than those obtained with open end catheters. According to this study the maximum urethral pressure — measured by microtip catheter with 200 ml saline at 37°C temperature in the bladder and the subject seated — in healthy women after the age of 25 is  $cm H_2O = \text{age} \pm 20 cm H_2O$ .

## ACKNOWLEDGEMENT

The author is indebted to Dr Per K Oystad, Norwegian Defence Research Establishment, Lyde, for help in determining the catecholamines in the urine.

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Submitted for publication May 9 1980

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## **ANNOUNCEMENT**

**The International Federation of Gynecology and Obstetrics  
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## NEUROGENIC BLADDER

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**Abstract** A survey of the innervation of the bladder and urethra is presented. Based on the defects of innervation the types of neurogenic bladder disorders are classified and described. The principal diagnostic procedures, cystometry and sphincter-electromyography, are reviewed in detail. The findings at voiding cysto-urethrography are discussed. It is important to be able to distinguish between voiding disorders of non-neurogenic and neurogenic origin. The specific treatment of the various types of neurogenic disorders of the lower urinary tract is described.

Bladder disorders in the female have traditionally been managed by gynecologists. In recent years a closer cooperation has been established between gynecologists and radiologists in the evaluation of a group of patients. Consultation with a neurologist is often indicated to evaluate a possible neurological lesion. This paper presents a short survey of bladder disorders that may be encountered in gynecological patients.

## INNervation

The innervation of the lower urinary tract consists of afferent and pathways within the central nervous system and peripheral neurons.

**Central nervous system** The principal micturition center is localized in the pontine mesencephalic area in the formation of the brain stem (6, 8). This center is responsible for the coordination of bladder function. It is influenced by nervous input from three major areas:

- 1. Cortical centers ensuring volitional control. These have facilitating as well as inhibitory effects on the brain stem; the inhibitory effects prevailing during continence.
- 2. Subcortical (and possibly other) centers important for the coordination of the detrusor muscle and the smooth musculature in the pelvic floor and urethra.
- 3. Afferent impulses, especially proprioceptive impulses, ensuring initiation and maintenance of micturition.

The sacral grey matter contains the motor nerve cells of the secondary micturition center, directly involved in the peripheral bladder, urethra and pelvic floor innervation. The sacral grey matter is controlled by the brain stem micturition center via the reticulospinal and pyramidal tracts (6, 8) (Fig. 1). The sympathetic outflow to the bladder and urethra is localized in the lower thoracic and upper lumbar spinal segments.

**2. Peripheral nervous system** The peripheral innervation is autonomic — both parasympathetic and sympathetic — as well as somatic (5) (Fig. 2).

**2a. Autonomic innervation** The principal efferent innervation of the detrusor muscle is parasympathetic, conducted through the pelvic nerves (nervi erigentes) (11), which are interrupted in synapses in the pelvic ganglia. The neuromuscular transmitter in the bladder wall is presumably acetylcholine, although other substances may be involved. The influence of the sympathetic innervation is still controversial (26). The afferent nerves, conducting extero- and proprioceptive impulses, are located within the same nerves with connection to the brain stem micturition center and the central cortex.

**2b. Somatic innervation** The striated musculature of the pelvic floor is mainly innervated by the pudendal nerve. However, recent research seems to indicate that the striated urethral sphincter is not innervated by the pudendal nerve, but through the pelvic nerves (9).

**Normal micturition** During bladder filling, the exteroceptive and proprioceptive input to the central nervous system increases with subsequent activation of the brain stem micturition center and cortex. At a certain bladder volume threshold, the person will experience a desire to void and a micturition reflex is triggered. This reflex can be voluntarily suppressed by the cortical centers. When micturition is socially convenient, the volitional inhibition of the brain stem center is reduced, and a coordinated micturition act is elicited via the secondary micturition center in the sacral grey matter.

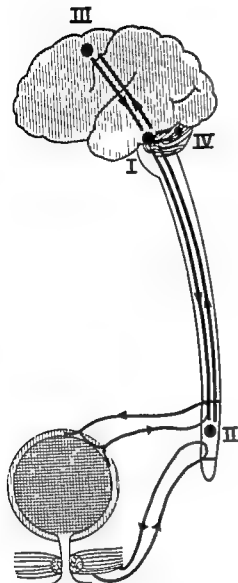


Fig 1 Schematic illustration of the innervation of the lower urinary tract

- I Principal micturition center in the brain stem
- II Secondary micturition center in the sacral spinal cord
- III Cortical centers for volitional control of micturition
- IV Cerebellar centers for coordination of bladder and urethral function

## CLASSIFICATION

From a clinical point of view neurogenic bladder disorders can be divided into two main groups depending upon whether the neurological lesion is located within (I) or outside (II) the central nervous system (4, 17). A third group comprises lesions of the sensory system (III) (Fig 3).

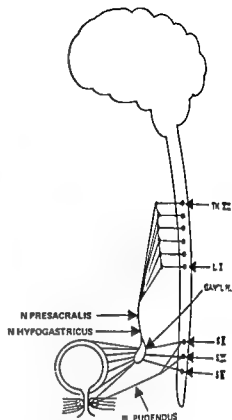


Fig 2 Schematic illustration of the peripheral of the lower urinary tract

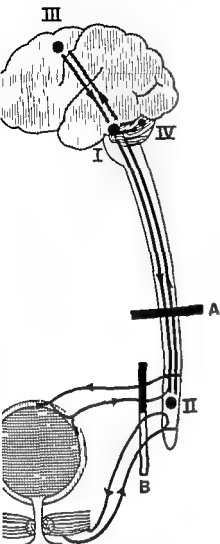
### I Upper motor neuron lesion (Supranuclear) paresis reflex bladder automatic bladder

This type of neurogenic bladder seen in lesions above the sacral spinal cord is characterized by preservation of a micturition reflex although the volitional control is impaired or completely abolished. Upper motor neuron lesions are seen in cerebral insults (24), multiple sclerosis (1), Parkinson's disease, spinal cord injury (4) and a variety of other conditions affecting the central nervous system.

### II Lower motor neuron lesion (Infranuclear) paresis flaccid bladder autonomous bladder

This type is characterized by absence of the micturition reflex. Lower motor neuron lesions are seen in lesions of the cauda equina and conus medullaris such as protruded lumbar disc (7, 10) and other conditions affecting the peripheral bladder nerves mainly following pelvic surgery (16).

In upper motor neuron lesion as well as lower motor neuron lesion the innervation of the floor may be impaired in the upper motor neuron lesion most frequently leading to pelvic floor spasm (detrusor sphincter-dyssynergia) whereas in lower motor neuron lesion the innervation of the floor is lost leading to pelvic floor flaccidity.



Classification of neurogenic bladder dysfunction according to location of neurological lesion  
 ○ = sensory neuron lesion  
 ● = motor neuron lesion

neuron lesion may be associated with pelvic paralysis. However, the type of bladder and bowel dysfunction may be dissociated. Peripheral neuropathy (Sensory lesion tabetic type).

Peripheral neuropathy is characterized by impairment of normal bladder sensation initially with preservation of the micturition reflex.

Compulsive lesions are typically seen as complications in diseases such as diabetes mellitus (12), pernicious anemia and tabes dorsalis.

## DIAGNOSIS

The diagnosis of the neurogenic bladder is based upon a thorough history, general physical examination, clinical neurological examination and cystometry with electromyography (EMG) from the sphincter apparatus.

**History** Two main points should be clarified:

1) *Vita ante acta* Genetic dispositions (e.g. diabetes), congenital disorders (e.g. meningocele), trauma (cerebro-spinal, pelvic), surgery (major pelvic surgery, neurosurgery), presence of neurological disease (e.g. multiple sclerosis, parkinsonism), gynecological and urological surgery and

2) *Actual urological problems* Time of onset of voiding symptoms related to age and *vita ante acta*. The micturition pattern should be analyzed with attention to frequency, nocturia, urgency, incontinence (stress/urge/continuous), difficulty in initiating micturition, straining, feeling of bladder emptying and pain. It is very helpful to have the patient fill in a micturition chart for a period of time (Fig 4). Information should be obtained about present medication with possible effect on bladder function, such as anticholinergic drugs. Attention should be given to possible rectal and sexual dysfunction.

Physical examination includes gynecological examination and a gross neurological examination. Special attention should be drawn to testing of the sacral reflexes: the *anal reflex*, where anal sphincter contraction is elicited by inserting a finger in the anal canal; the *ano-cutaneous reflex*, consisting of anal sphincter contraction — elicited by pin pricking the perianal area; and the *bulbo-cavernosus reflex* in

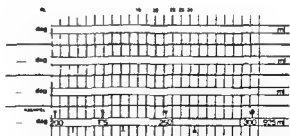


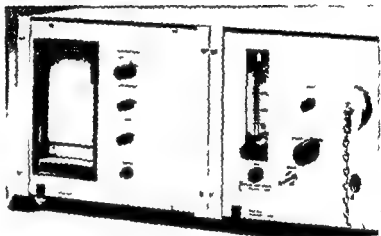
Fig 4 Micturition chart for registration of voiding events during a 3-day period. The numbers indicate the voided volumes.

▽ = Normal voiding, ▽ = Dysuria, ▹ = Severe pain during voiding, | = Incontinence episode, ⊥ = Change of underwear due to incontinence, ⊥ = Severe incontinence episode, — = Sleep period.





Fig 5 Cystometers  
A. Lewis recording cystometer for traditional water cystometry  
B. DISA type 21G45 cystometer for CO<sub>2</sub>-cystometry



such anal sphincter contraction is triggered by gently squeezing the glans penis/clitoris or pulling on a urethral balloon-catheter. Furthermore the sensory innervation of the saddle area should be tested.

**Cystometry** Cystometric investigation can be regarded as the reflex hammer used for testing the micturition reflex. The main purpose of performing cystometry is testing for detrusor reflex activity. This test may demonstrate presence or absence of detrusor contractions and the patient's ability to actively control these contractions.

Other important information gained from cystometry is the presence or absence of bladder sensation. Cystometry can be performed using either water (23) or carbon dioxide (3) as the filling medium (Fig 5).

After emptying the bladder the patient is catheterized transurethraally and the residual urine volume is measured. The bladder is filled continuously while intravesical pressure is simultaneously recorded. The volume at which first sensation of fullness is experienced is recorded (FS) and the patient is in-

structed to suppress voiding. Filling is continued until the patient feels discomfort. The presence of detrusor contractions with an amplitude exceeding 15 cm H<sub>2</sub>O during filling is registered as *uninhibited detrusor contractions* (21). If uninhibited detrusor contractions are not registered during filling the patient is instructed to void in order to see if a micturition reflex can be demonstrated. The patient is then asked to inhibit this reflex in order to test the voluntary control.

The voluntary control is further tested by the detrusor reflex activating procedures such as cough-

ing with a full bladder, repeated cystometry while standing or walking positions (27).

Testing of the pelvic floor innervation and its coordination between detrusor and pelvic floor necessitates the use of simultaneous sphincter electromyography. This is most easily performed using a ring electrode mounted on the urethral (28) (Fig 6).

**A normal cystometry and sphincter activity** is shown in Figs 7–8. During bladder filling the sensation of fullness is usually experienced at a volume of 133–215 ml (34). Normal sensation is an important sign of intact parasympathetic innervation. The maximum cystometric capacity is reached when the patient has a strong desire to void. Cystometry is performed with the patient relaxed, 'holding on' in order to test sphincter control. **Upper motor neuron lesion** (Figs 9 & 10) is characterized by uninhibited detrusor contractions. First sensation — if present at all — is experienced at a low bladder volume and is often



Fig. 1. Ring electrode for urethral electromyography (DISAronics) mounted on a 16 F Foley bag catheter.

by urgency and the onset of uninhibited detrusor contractions. Fig. 9 shows the findings in a patient with normal sphincter control, whereas Fig. 10 shows detrusor sphincter dyssynergia characterized by absence of voluntary control of the sphincter function and increasing sphincter contractions during detrusor contraction.

**Symptoms of upper motor neuron lesion.** Frequent urgency, nocturia, urge incontinence as seen in spinaesthesia and cerebral vascular insults. In patients with detrusor sphincter dyssynergia, further urgency and impaired bladder emptying are seen in detrusor sphincter dyssynergia as seen especially in spinal cord injury, but also in patients with multiple sclerosis.

**Lower motor neuron lesion.** The cystometrogram is characterized by impaired or absent bladder sensation, a fairly large bladder capacity and absence of detrusor reflex activity. Absence of a detrusor reflex during cystometry may be indicative of a lower motor neuron lesion, but the final diagnosis requires testing for denervation supersensitivity (15).

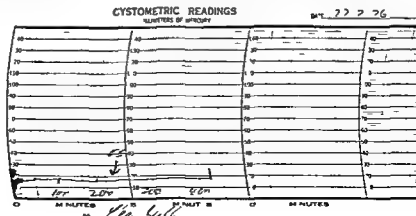
In lower motor neuron lesions after pelvic surgery, the pelvic floor innervation is usually intact (Fig. 11), whereas lower motor neuron lesions due to lesions of the cauda equina often are associated with pelvic floor paralysis (Fig. 12).

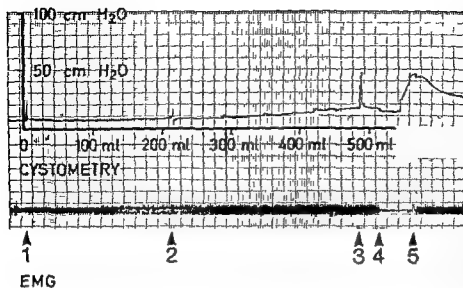
**Main symptoms of lower motor neuron lesion.** The symptoms depend upon whether the pelvic floor innervation is intact. When it is intact, there are often few symptoms over a long period of time. When symptoms occur, these are often due to a large capacity bladder with high residual urine retention or overflow incontinence. When the pelvic floor innervation is damaged, severe continuous dribbling incontinence prevails.

**Peripheral neuropathy.** This lesion is cystometrically characterized by unpaired sensation and a large bladder capacity. Detrusor reflex activity is present in the early phases, but may be impaired or abolished in the later stages.

**Main symptoms.** Few symptoms are present until the patient presents with decompensation of the bladder. At this stage, urinary retention eventually with overflow incontinence dominates.

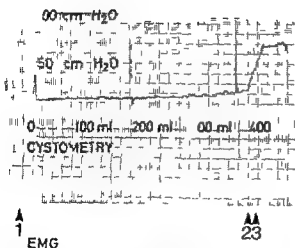
**Radiological examination.** Radiological examination is not carried out in order to establish the diagnosis of neurogenic bladder dysfunction. Indications are evaluation of ureteral reflux, bladder and urethral diverticula, calculi, bladder outflow conditions and the relation of the bladder to its supporting structures.





**Fig 8** Normal  $\text{CO}_2$ -cystometry and urethral electromyogram

- 1 Start of bladder filling
- 2 First sensation at a bladder volume of 210 ml
- 3 Cystometric bladder capacity of 490 ml. The patient asked to cough and void. Filling stopped.
- 4 Patient asked to void. Urethral electromyogram completely and a few seconds intravesical rises
- 5 Patient asked to void. Urethral gram again shows some bladder pressure starts to increase



**Fig 9**  $\text{CO}_2$ -cystometry and urethral electromyogram from a patient with multiple sclerosis and upper motor neuron lesion with normal sphincter control

- 1 Voluntary squeezing before start of bladder filling
- 2 Uninhibited detrusor contraction starts
- 3 Patient asked to relax. The disappearance of electromyographic activity during the rising phase of the detrusor contraction confirms voluntary control of the sphincter



**Fig 10**  $\text{CO}_2$ -cystometry and urethral electromyogram from a patient with a transverse spinal cord lesion at T7 and per motor neuron lesion with sphincter dysynergia. At a bladder volume of 100 ml an uninhibited detrusor contraction is elicited. At the same time urethral sphincter activity increases markedly. The patient is not able voluntarily to relax the urethral sphincter confirming a detrusor-sphincter synergy

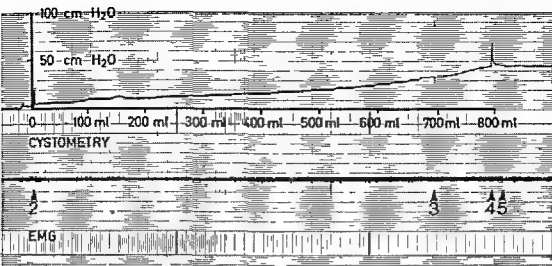


Fig 11 CO<sub>2</sub>-cystometry and urethral electromyogram from patient with a lower motor neuron lesion and intact pelvic floor after radical hysterectomy. The patient voluntarily squeezes before start of bladder filling. The patient has no sensation at a bladder volume of 700 ml.

4 Cystometric bladder capacity of 800 ml. Patient asked to cough and bladder filling stopped.

5 Patient asked to void without straining. The patient can only shortly relax the sphincter and no detrusor contraction is elicited.

A similar cystometrogram is seen in patients with neuropathic lesions.

Nevertheless neurogenic dysfunctions may be suspected from voiding cysto-urethrograms (29). In patients with lower motor neuron lesions present a rounded bladder with serrated mucosal pattern as signs of detrusor overactivity even on the initial film of the supposedly normal bladder. Cough picture may show active voiding. Micturition pictures will show either relaxation of the urethral sphincter or dilatation of the posterior urethra due to spastic sphincter. detrusor sphincter dyssynergia. Trabeculation and diverticula are common (Fig 13).

In patients with lower motor neuron lesions may be suspected from large bladder volume and/or flaccid appearance of the bladder at rest. Voiding takes place without contraction of detrusor function but with abdominal pressure. Squeezing the bladder against the pelvic floor. Large volume urinary retention is common (30) (Fig 14).

In patients with peripheral neuropathy may show flaccid bladders at rest like the lower motor neuron lesion but voiding may show rounding of the bladder and serration of bladder mucosa as signs of detrusor function in the early cases. Insufficient bladder emptying is the rule (13) (Fig 15).

Neurogenic bladder in gynecology. The complex problem involved in recognition of neurogenic bladder and its management are of great importance to gynecologists.

There are two reasons for this. First major pelvic surgery such as radical hysterectomy will often lead to lower motor neuron lesions (16). Secondly among patients referred for urinary incontinence a significant number will have neurogenic bladder dysfunction.

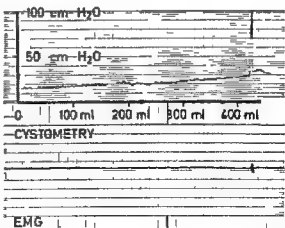


Fig 12 CO<sub>2</sub>-cystometry and urethral electromyogram from a patient with traumatic cauda equina lesion and lower motor neuron lesion including a flaccid pelvic floor. The patient has no sensation to void and urethral electromyogram is completely silent. The filling is stopped at 400 ml due to discomfort.



**Fig 13** Upper motor neuron lesion. Picture at rest. Bladder contracted, rounded with serration above the trigone as signs of uninhibited detrusor contraction. Contrast medium added in the vagina.

tion. It is important to distinguish between the true upper motor neuron lesion and simple urge incontinence. The clinical picture may be identical and the diagnoses rest on the urodynamic and neurological evaluation.

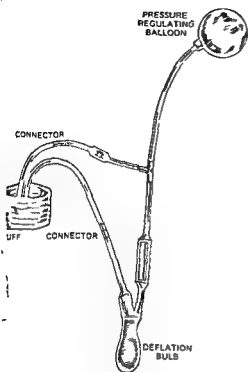
In a prospective study in Copenhagen county over a two year period, 54 out of 369 patients had unin-

**Fig 14** Lower motor neuron lesion. Picture at rest. Bladder low position.

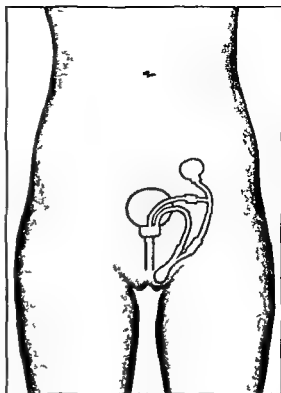
hibited detrusor contractions during cystometry. Of these 54 patients, 20 had overt neurological disease. In 3 of the patients, the cystometric uninhibited detrusor contractions was the first sign of multiple sclerosis.



**Fig 15** Sensory bladder lesion. a) Picture at rest, large bladder, normal position. b) During voiding, slight rounding and faint serration over the trigone as signs of detrusor contraction.



III Schematic illustration of the Scott Bradley Timm sphincter prosthesis model 742 a) The cuff is implanted around the bladder neck the bulb in the labium majus and the pressure regulating balloon is placed intraperitoneally. The cuff keeps the urethra compressed. By squeezing the



bulb the cuff is emptied into the pressure regulating balloon and the patient is able to void. The cuff is refilled automatically after 2 minutes from the pressure regulating balloon. b) The artificial Scott Bradley Timm sphincter placed in situ.

## TREATMENT

Lower motor neuron lesions are characterized by uncoordinated detrusor contractions. This condition is treated pharmacologically using parasympatholytic drugs such as emepronium bromide (Cetsprin<sup>(R)</sup>) (19) or methantheline bromide (Banthine<sup>(R)</sup>) (33). Then the treatment is contraindicated or fails, resection of the inferior hypogastric plexus or sacral nerve block (20) sacral nerve blockade or sacral nerve resection (32) come into consideration. If there is a possible bladder outlet obstruction due to pelvic floor spasticity should only be treated when leading to a red bladder emptying and recurrent urinary infections. The treatment is urethrotomy using the external (25) or endoscopic external sphincterotomy (14). In some patients treatment is ineffective and should be supplemented by external collection devices (31). The function of these are however

poor in the female therefore some cases are definite candidates for urinary diversion procedures. Lower motor neuron lesions. When the pelvic floor innervation is intact the main treatment is by instruction in bladder retraining. Because of the few symptoms it is important to diagnose this condition before the patients develop a large capacity bladder with residual urine. The treatment consists of instruction in voiding at regular time intervals (every 3 hours during day time) bladder emptying by straining and use of the Crede manoeuvre.

If the pelvic floor innervation is damaged with subsequent severe incontinence implantation of an artificial urinary sphincter should be considered e.g. the Scott Bradley Timm sphincter (18).

Peripheral neuropathy is treated in the same way as lower motor neuron lesions. If these patients have developed a large bladder capacity and/or residual

urine the treatment should be supplemented by a parasympathomimetic drug (e.g. Carbacholine) (22)

In the treatment of neurogenic bladder disorders urinary diversion or permanent catheter drainage may eventually be inevitable but should only be used as ultimum refugium

The follow up of patients with neurogenic bladder dysfunction is of great importance. The main risk in these patients is the development of progressive renal failure. Therefore their management should include regular urine cultures and evaluation of renal function

### ACKNOWLEDGEMENTS

This work was supported by the Danish Foundation for Medical Research, Region of Copenhagen, The Faroe Islands and Greenland

We want to express our thanks to Mrs Margrethe Maibell for typing the manuscript

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*Submitted for publication November 2 1978*

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## ANNOUNCEMENT

**The Pan American Congress of Andrology** will take place in Mexico City Americana Fiesta Palace Hotel January 26-31 1981

*Informations requests for registration and travel information should be directed to*

Dr A Negro-Vilar

University of Texas Health Science

Center at Dallas

Southwestern Medical School

Department of Physiology

5323 Harry Hines Boulevard

Dallas Texas 75235

USA

*Informations requests for registration and travel information should be directed to*

Gerald Magazinski

Congress Administrator

31600 West Chicago

Livonia Michigan 48150

USA

**The Third World Congress of Human Reproduction** is to be held in West Berlin March 22-26 1981

*Main topics*

- Central nervous system and regulation of reproduction
- Surgical and morphological aspects of reproduction in men and women
- Gonadotrophins and their target tissue
- Beginning of life *in vivo* and *in vitro*
- Experimental embryology of mammals
- High risk reproductive factors in breast and genital cancer
- Influence of the environment and drugs on reproduction
- Free Communications
- Film Festival

*Information*

Congress Secretariat

Priv Doz Dr L Mettler

Frauenklinik der Universität

Hegewischstrasse 4

D-2300 Kiel 1

West Germany

**The Third International Congress on the Menopause** will be held in Belgium at the coastal resort of Oostende 9-12 1981 just before the holiday season begins.

This Congress will differ from the previous ones in Grande Motte 1976 and in Jerusalem 1978) in that as the aim in the past was to arrive at a conclusion this time we shall bravely attempt to confront controversies. It will however take the same format — a number of small workshops rather than a meeting attended by everyone.

*For further information*

The International Menopause Society

8 av Don Bosco

B 1150 Brussels

Belgium

Tel (02) 771 98 98 and (02) 771 96 45

**The Eighth Asian Congress of Obstetrics and Gynaecology** will be held in Melbourne in October 1981

An exciting and varied programme will be presented including expert plenary speakers from Asia Australia and other Centres. The Congress themes include

- Population Control
- Maternal and Perinatal Mortality
- Trophoblastic Disease
- Gynaecological Malignancy
- Obstetrical and Gynaecological Endocrinology

The official language will be English  
*Further information may be received from*

The Organizing Secretary

VIIIth Asian Congress of

Obstetrics and Gynaecology

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Victoria

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## TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA WITH LOCAL EXCISIONAL BIOPSY AND CRYOSURGERY

Svein Erik Tronstad and Rolf Kirschner

From the Department of Obstetrics and Gynecology Central Hospital Skövde Sweden

One hundred and ninety-eight patients with abnormal cytologic smears had extensive diagnostic biopsy of portio combined with endocervical curettage. Severe dysplasia or cancer *in situ* was found in 57 per cent of the patients. During a period of observation of 6-42 months (mean 18 months) the cervical intraepithelial neoplasia (CIN) regressed in 54 per cent. Cryosurgery was used to treat 93 patients with persistent CIN after biopsy. Treatment was successful in almost 70 per cent after one single application of cryosurgery. The complications were negligible. Treatment was less successful in increasing histological severity as well as with increasing age of the patients; this applies both to biopsy and to surgery. Cryosurgery is significantly less effective when CIN is of both ecto- and endocervical origin. Extensive multiple biopsies of the portio in combination with endocervical curettage performed under general anesthesia followed by cryosurgery with persistent cytological abnormalities are indicated as an alternative to conization, especially in younger patients with ectocervical

Colposcopy together with guided excision biopsy and cervical curettage is gaining in popularity (1-7, 12). It is a small procedure which can be performed on outpatients and it has been reported to have good results both in diagnosis and treatment (1, 2, 3, 19, 25). The last decade has seen the introduction of cryosurgery for the treatment of CIN, usually of mild to moderate degree and especially when sited on the ectocervix (3, 4, 6, 10, 21, 22, 23, 24).

We decided to investigate

- The curative effect upon CIN of excisional biopsies of the cervix and
- Cryosurgery as an alternative method to conization in the treatment of CIN

## METHODS

Since 1974 the following regimen for control and treatment of CIN has been used at the Gynecological Department Central Hospital Skövde Sweden (Fig. 1).

On finding abnormal smear group III or more (Cytologiskt Laboratorium Malmö Sweden) the patient is referred to histological diagnosis by cervical curettage and extensive multiple biopsies of the portio. The operation is done under general anaesthesia on outpatient basis and is guided but not decided by a Schüller test (19). The biopsies are performed with a Hartmann's conchotome (Medicon West Germany) with a diameter of 9 or 11 mm and a depth of excision of 3 mm (Fig. 1). Multiple overlapping biopsies extending 3 to 5 mm beyond the margin of the nonstained area together with endocervical curettage give sufficient removal of tissue for a reliable histological examination. Electrocautery is used when necessary to control bleeding.

In the age groups above 45 years or when abnormal uterine bleeding is present, cervical dilatation and curettage of the corpus is added to the procedure.

After surgery the patients are checked with a smear every three months during the first year, then less frequently (Fig. 1). Patients are registered in a special filing system which ensures the continuous follow-up. Failure to attend results in a reminder being sent. Very few patients are lost to follow-up. Patients showing persistent cytological abnormalities within one year after the above procedure are treated by cryosurgery. The majority of persistent abnormalities are detected at the first smear after biopsy. Recurrences seen

the introduction of the Papanicolaou smear three decades ago, the number of patients, especially younger ones, with a diagnosis of cervical intraepithelial neoplasia (CIN) has steadily increased (20, 25). Lately the incidence in Sweden has been 7 000-9 000 new cases per year. Various methods for clinical control and treatment of CIN have been advocated, from the radical step of immediate hysterectomy to that of frequent smear control. For patients with severe dysplasia and carcinoma *in situ*, surgical conization is a very common method of treatment today (2, 9, 11, 15, 17). Conization has disadvantages in the form of immediate surgical as well as late complications, which may be of importance for future fertility and bearing (5, 9, 11, 13, 14). Necessitating conization also increases the demands on the gynecological department. These disadvantages have led to the present indications for conization have been questioned (16).

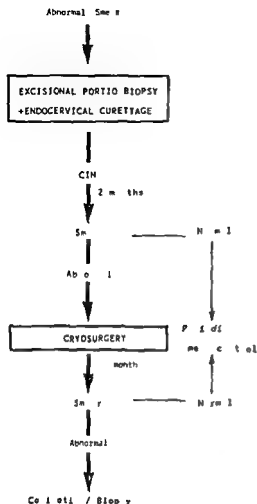


Fig 1 Regimen for control and treatment of CIN

after one year receive further curettage and biopsy in order to check the histological diagnosis and to exclude microinvasive or invasive cancer before further treatment is instituted.

Cryosurgery is done as an outpatient procedure without analgesia. A Spemby PCG 12 N<sub>2</sub>O driven cryo unit is used that produce -80 °C at the cryodes (Fig 2). The cryodes

Table 1 Results of cryosurgery 91 patients

Diagnosis	Number	Healed	
		Number	Per cent
Cancer in situ	18	28	93
Severe dysplasia	30	23	76
Moderate dysplasia	11	10	90
Mild dysplasia	4	3	75
Total	93	64	68

are interchangeable: a conic tipped cryod is used exclusively because it fits well into the whole cervix. A firm contact between the tissues and the cryodes is necessary for maximum freezing effect. This is facilitated by retaining Cusco-speculum in the vagina and two Schöfer forceps holding the cervix at 3 and 9 o'clock. The duration of the procedure is decided by observing the well demarcated edge of the frozen tissue on the portio. Usually the application of the cryodes will freeze the cervical tissue to 10 mm.

To begin with we used the single freeze technique. Subsequently a double freeze technique was used as reports suggested that better results were achieved by this technique. Thus the procedure was repeated after 20 min.

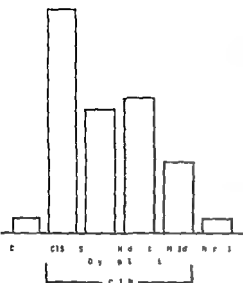
The procedure has few side effects and does not necessitate time off work. After four months the patients enter the follow up system already described with persistent smear abnormalities after cryosurgery treated individually taking into account factors such as histological severity, progression and the site and size of the lesion as well as age and parity and other gynecological or other complaints. Further cryosurgery is rarely performed: most patients receiving cold treatment

## MATERIAL

**Biopsy.** During the period from May 1st 1974 until 1977 198 patients were treated by curettage and portio biopsy. Ages range from 17 to 64, the median age 35 years. Histologically 57 per cent of the lesions were classifiable as severe dysplasia or cancer in situ (Fig 1).



Fig 2 Instruments used in our regimen: Spemby PCG 12D cryo-unit with cryodes, Bivalve Cusco self-retaining vaginal speculum, Hartmann conchotomes diameter 9 and 11 mm, Schröder forceps.



J Histological diagnosis 198 patients

5 patients histology were benign and in 5 patients we had invasive cancer. These 10 patients were excluded from the 188 patients.

The time of observation was from 6 to 42 months, averaging 18 months.

Ninety three patients were treated with surgery. These consisted of 62 patients from our own series and in addition 31 patients referred by other gynecologists who were treating the patients according to regimen. Histologically 88 per cent had severe dysplasia or cancer (Table I).

The time of observation was 7 to 36 months, averaging 18 months.

## RESULTS

Subsequent smears were normal in 101 (53.7 per cent) of the 188 patients with CIN (Table II).

Out of the remaining 87 patients showing persistent histological abnormalities 82 underwent cryosurgery.

Table II Results of multiple biopsy 188 patients

Diagnosis	Number	Healed	
		Number	Per cent
in situ	16	34	44.7
severe dysplasia	42	19	45.2
moderate dysplasia	46	30	65.2
dysplasia	24	18	75.0
Total	188	101	53.7

Table III Results in relation to the site of the lesion in 78 cryosurgically treated patients with CIS and severe dysplasia

	Ectocervical lesion		Ecto- and endocervical lesion	
	Number	Per cent	Number	Per cent
Healed	45	70	6	43
Non healed	19	30	8	57
Total	64	100	14	100

To these were added the 31 patients referred by other gynecologists. Of these 93 patients 64 (69 per cent) had normal smears after one treatment. Normal smears were found less often after both biopsy and cryosurgery in the patients with more severe histological lesions (Tables I and II).

The results were considerably better for ectocervical lesions compared to those who also had endocervical changes. The difference is statistically significant with  $p < 0.05$  (Table III).

After cryosurgery the results were better in the younger age groups: the patients with normal smears averaging 31 years of age and those showing persistent CIN after cryosurgery averaging 36.5 years.

**Complications after excisional biopsy and curettage** are hemorrhage and infection. Two patients had hemorrhages of approximately 300 ml necessitating extra sutures in one, tamponade and observation for 24 hours in the other. Nine patients had postoperative pelvic inflammatory disease (PID) of moderate degree, all showing resolution within two weeks on conventional therapy.

**Complications after cryosurgery** are very few: no hemorrhage or cervical stenosis have been seen so far. One patient was admitted to hospital and was given antibiotics for a severe lower genital tract infection (LGTI). Laparoscopy was performed and PID was excluded. Retrospectively the patient had had clinical cervicitis at therapy; this should have contraindicated cryosurgery.

A side-effect rather than complication is the copious clear discharge which most patients experience after cryotherapy. Another side-effect is the short flushing that 60 per cent of the patients experience during the thawing of the frozen tissues. It usually involves the upper trunk and extremities in a wave form and there may be a transient headache. This may be caused by release of histamin from the

damaged cells. Apart from the immediate discomfort above mentioned, this has not been associated with any long term problems or other serious side effects.

## DISCUSSION

In Sweden we have a conservative attitude to the treatment of the uterine cervix. The most common method for treatment of severe dysplasia and carcinoma *in situ* is a diagnostic and therapeutic conization, sometimes as the first measure after an abnormal cytological smear. This method is very effective but can lead to both direct and late complications and necessitates hospitalization.

The Swedish nation wide cytological screening programme and the frequent screening of younger women outside the programme has resulted in a sharp increase in the diagnosis of asymptomatic CIN.

The peak age incidence for cancer *in situ* and dysplasia (CIN) is between 30 and 40 years (8) and is decreasing (6, 7, 20). These facts justify our efforts to find alternative methods of treatment for the young fertile woman, methods combining therapeutic efficiency and the accurate exclusion of invasivity with few complications and minimal danger to fertility.

Our technique using general anaesthesia provides a large and more satisfactory sample of tissue for pathological analysis than when the procedure is done with the patient awake. Diagnoses should be more reliable both for ectocervical as well as for endocervical changes. The risk of missing early invasive angles is minimized. The thorough histological staging obtained is of great assistance on the further optimal treatment of the patients.

We get a primary therapeutic effect in half of our patients. This corresponds well with other authors (1) and shows how important it is to check the therapeutic effect of biopsies by taking follow up smears before resorting to further treatment. We consider a primary conization based on only one cytological abnormalities an unnecessarily large procedure which should be replaced by multiple excisional biopsies.

Our regimen differs from other studies employing cryosurgery (3, 21, 23, 24). The therapeutic effect of alternative treatment of CIN is difficult to assess because biopsy will change the natural course of the disease. This means that if biopsy and cryosurgery are performed together it is not possible to measure the therapeutic effect of the two components of treatment. In our series of 188 patients, 101 patients had

normal cytology after biopsy alone and cryosurgery would have been performed in these cases had the cervix not been reassessed after the biopsies. Criticism of the cryosurgical treatment of severe dysplasia and CIS has occurred largely because tissue for histological examination is not available at the time of treatment. The time interval between biopsy and the first post biopsy smear in our study is 2 to 4 months. The total time interval between the initial abnormal smear and the first smear after cryotherapy is 6 to 8 months. Progression to invasive cancer in such a short interval is very rare. Nevertheless we do not perform repeated cryotherapy for persistent cytological abnormalities but prefer conization as this provides an accurate histological diagnosis. For this reason our success rate for cryotherapy is less than that of other published works where repeated cryotherapy obtain a success rate of up to 72 per cent (4, 10, 21, 23, 24). It should be noted that these results are often the effects of biopsies plus repeated cryosurgery.

When comparing our regimen of treatment with conventional conization we have a lower rate of healing (2). This is especially true for older patients and patients with endocervical lesions or a combination of both. Conization however is a much more complex procedure involving a stay in hospital, a higher rate of complications and the need for post operative restrictions on activity. Also post operative smears are as important after conization as after excisional biopsy and cryosurgery.

## CONCLUSION

By doing multiple extensive biopsies of the cervix combined with cervical curettage under general anaesthesia on an outpatients basis in patients with cytological CIN we obtain a reliable histological diagnosis as well as a satisfactory therapeutic effect in more than half of the patients.

In patients with persistent cytological abnormalities and CIS after multiple biopsies cryosurgery is useful especially on younger patients with ectocervical lesions before resorting to other methods of treatment.

## ACKNOWLEDGEMENTS

This research was supported by the Medical Board of the Council of Skaraborg, Sweden.

The authors wish to thank Ass. Professor G. Ivarsson for reading and revising the manuscript.

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Submitted for publication December 29 1978

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Annual Subscription 17 50 pounds U.K. and EIRE 22 50 pounds Overseas US dollar 60 00

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# IN VITRO INDUCTION OF MURINE SUPPRESSOR T CELLS BY HUMAN CHORIONIC GONADOTROPIN

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Human chorionic gonadotropin (HCG) has previously been considered to be immunosuppressive and by prohibiting maternal rejection of the fetal plant to be one of the factors responsible for the successful outcome of pregnancy. The mechanism by which it exerts its pregnancy retaining effect is however as yet unknown. The present study shows that HCG has the ability in mice of inducing lymphocytes which are sublethally competent to depress a polyclonal antibody response induced by different B cell mitogens. It is therefore suggested that HCG may exert its fetoprotective action by inducing suppressor T cells. These lymphocytes would thus be the activation of cells which are responsible for placental rejection.

recognized in a variety of systems where they have been shown to impair B lymphocyte responses as induced by antigens (6) or polyclonal B cell activators (21-22). The immunosuppressive cross reactivity between HCG on lymphocytes from other species including mice (2, 7, 11, 14) indicates that animal systems may be used for evaluating the mechanism underlying the suppressive effect of HCG. In this paper we describe the induction of suppressor T cells in mice by HCG. The possible importance of these observations and their clinical implications are considered.

A mechanism which prevents the fetus, a histoincompatible graft from being rejected, is still an unsolved problem and although a vast number of explanations have been proposed, none has so far proved conclusive. Human chorionic gonadotropin (HCG), a glycoprotein produced in large amounts by syncytiotrophoblastic cells (4) has been shown to suppress mitogen induced proliferation of both T lymphocytes (1, 5, 11) and B lymphocytes (2, 10, 25). In these studies it has therefore been suggested that HCG is one of the main factors contributing to immunosuppression responsible for the survival of a fetal transplant. The mechanism by which it exerts its suppressive effect is however still unknown.

Human fetal protein synthesized in large amounts in the fetus is another hormone which is suggested to possess immunosuppressive properties (24). This suppressive effect has been claimed to be due to induction of suppressor T cells (17). This cell type was first described by Gershon and coworkers (9) and suggested that certain T cells were involved in the depression of a sheep red blood cell specific immune response. To date suppressor cells have been

## MATERIAL AND METHODS

**Animals.** Female C3H/Tif mice used at the age of 8-12 weeks were bred and maintained in our animal colony. **Cells.** Spleens were removed aseptically and cell suspensions were prepared by pressing the tissue through a steel mesh into a balanced salt solution (BSS). After brief sedimentation the non sedimented cells in the supernatant fluid were washed with BSS, resuspended in culture medium, counted and adjusted to appropriate concentrations. The viability was determined by trypan blue exclusion. **Culture medium.** Mishell Dutton medium was prepared from components purchased from Flow Laboratories Irvine, Scotland and was supplemented with HEPES buffer. All media were supplemented with 5 per cent heat inactivated human AB serum. **Mitogens.** Concanavalin A (Con A) was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 was obtained from professor T. Holme, Dept. of Bacteriology, Karolinska Institute, Stockholm, Sweden. Purified protein derivative of tuberculin (PPD) was purchased from Statens Serum Institut, Copenhagen, Denmark. **Assay of antibody synthesis.** Antibody production was determined using a modification (21-22) of the original mouse plaque assay system (13). Cells were harvested with a plastic spatula after 2-3 days of culture, washed twice in BSS and resuspended in 1 ml of BSS. 0.5 ml of 0.5 per cent agar (Difco Laboratories, Detroit, Michigan, USA) in BSS



Table I Suppressive effect of HCG activated cells

Exp No	Substance used for preactivation	Mitogen used for activation	IgM anti NNP PFC/cult	Suppression per cent
1	0	LPS	1 375	0
		PPD	1 005	0
	Con A	LPS	40	97
		PPD	4	99.6
	HCG	LPS	450	66
		PPD	40	97
2	HCG (2x10 <sup>6</sup> dead precul cells)	PPD	1 050	-4
		PPD	1 050	-4
3	0	LPS	160	0
		PPD	4	98
	Con A	LPS	16	90
		PPD	16	90
	HCG	LPS	25	98
		PPD	55	96
4	0	LPS	800	43
		PPD	790	47
	Con A	LPS	235	0
		PPD	13	94
	HCG	LPS	145	38
		PPD	145	38

Spleen cells from female C3H/Tif mice were cultured for 48 hours in the presence of 10 µg Con A/ml or 2 000 IE/ml HCG. Thereafter the cells were harvested and 2x10<sup>6</sup> preactivated cells were mixed with 8x10<sup>6</sup> syngeneic fresh spleen cells. The admixture was cultured in the presence of 100 µg LPS/ml or 100 µg PPD/ml and assayed on day 3 for the number of anti NNP plaque forming cells. In experiment 1 freeze killed cells were also included as a control.

containing 0.5 per cent DEAE dextran (Pharmacia, Uppsala, Sweden) was added to 3 ml tubes and kept at 4°C in a water bath. 0.05 ml of target erythrocytes heavily coupled with NNP (4-hydroxy 3,5 dinitrophenacetyl) diluted 1:8 in BSS, 0.2 ml of the lymphocyte cell suspension and 0.05 ml of guinea pig complement diluted 1:4 in BSS were added to each tube. The ingredients were mixed and evenly spread on 9 cm plastic Petri dishes and incubated for 3 hours at 37°C. The plaque forming cells (PFC) were counted using indirect light.

**Nylon wool column filtration.** T cell separation was performed using a modification of the method described by Juhus *et al.* (12). Briefly, spleen cells were added directly on nylon wool columns. 0.6 gram of the wool (Leukopak Leukocyte Filters LP 1, Fenwall Lab, Chicago, USA) was loosely packed into 10 ml syringes (20). The cells were eluted with BSS supplemented with 5 per cent fetal calf serum. Hormones. Purified HCG preservative free was generously supplied by Leo AB, Hålsingborg, Sweden.

#### Experimental procedure

Lymphocytes were cultured for 48 hours in the presence of 10 µg/ml of Con A or various concentrations of HCG. The cells were then harvested, washed in BSS and added in different ratios to fresh spleen lymphocytes. The cell mixture was either left untreated, stimulated with LPS (100 µg/ml) or with PPD (100 µg/ml). The number of IgM anti NNP SRBC secreting cells was determined on day two or three. The cultures were kept on a rocking platform, a procedure shown to increase the suppressive capacity (9).

Table II Effect of different HCG concentrations on the induction of suppressor cells

Substance used for preactivation	Mitogen used for activation	Exp 1 IgM anti NNP PFC	Exp 2 IgM anti NNP PFC
0	LPS	1 400	
	PPD	1 500	
Con A 10 µg/ml	LPS	40	
	PPD	60	
12.5 IE HCG	LPS	1 040	
	PPD	1 160	
250 IE HCG	LPS	615	
	PPD	800	
1000 IE HCG	LPS	N T	
	PPD	N T	
2000 IE HCG	LPS	800	
	PPD	790	

Spleen cells from female C3H/Tif mice were cultured in the presence of various concentrations of HCG. The cells were harvested and 2x10<sup>6</sup> preactivated cells were mixed with 8x10<sup>6</sup> syngeneic fresh spleen cells. This admixture was cultured in the presence of LPS or PPD in a concentration of 100 µg/ml and assayed on day 3 for the number of anti NNP plaque forming cells.

N T = Not tested

## RESULTS

**HCG induced suppressor cells.** Since it has previously been shown that 2 000 IE of HCG/ml is necessary to suppress the ability of the hormone used in concentration to induce suppressor cells was determined. As can be seen in Table I, preactivation of splenocytes with HCG resulted in the formation of cells which were capable of inhibiting the response of cells activated with lipopolysaccharide or with protein derivative. The inhibitory capacity varied from 38–97 per cent, possibly reflecting biological variability. Control cells not preactivated with Con A or HCG did not normally show a significant magnitude of the response (data not shown). In a second set of experiments various concentrations of HCG were tested for the induction of suppressor cells. As can be seen in Table II, a concentration of 250 IE/ml was sufficient to induce inhibition in this test system, since lower concentrations of HCG were found to induce inhibition in this test system as reported in our previous studies. It seems as if this preactivation assay is more sensitive for the detection of suppressor cells.

**Cell requirements for HCG induced suppressor cells.** To assess the putative T cell character of the HCG induced suppressor cells, nylon wool column filtered spleen cells which are normally highly enriched in T cells were tested for their suppressive capacity. Cells were either left untreated or preactivated with Con A or HCG.

## Table III Suppressive effect of HCG column purified T cells

Stimulans used for preactivation	Mitogen used for preactivation	IgM anti NNP PFC/cult		Suppression (per cent)	
		Spleen cells	T cells	Spleen cells	T cells
1	0	N T	1 300	N T	0
	PPD	N T	1 350	N T	0
	Con A	N T	11	N T	99
	PPD	N T	30	N T	98
2	HCG	N T	700	N T	47
	PPD	N T	710	N T	11
	0	1 200	1 400	0	0
	PPD	1 035	1 100	0	0
3	Con A	125	50	90	96
	PPD	250	45	76	96
	HCG	470	750	61	47
	PPD	640	600	39	46

cells or column purified T cells from female C3H/Tif mice were cultivated for 48 hours in the presence of 2 000 IE of HCG/ml. After the cells were harvested and  $2 \times 10^6$  preculivated cells were added to  $8 \times 10^6$  syngeneic fresh spleen cells. This admixture was cultured in the presence of LPS or PPD in a concentration of 100 µg/ml. Cultures were harvested on day 3 and tested for the number of anti-NNP plaque forming cells.

for two days. As seen in Table III these cells are capable of inducing a marked suppression of antigen induced B cell differentiation. Cytotoxicity does not seem to be involved, since cell survival was impaired in cultures containing suppressor cells (data not shown). Furthermore, the immunosuppression did not depend upon transfer of HCG from the primary culture, since freeze killed cells mixed with HCG did not diminish the PFC response of secondary cultures (Table I). We also investigated the effect of transfer of various amounts of cells preculivated with HCG and, as shown in Table IV, a ratio of at least 1:10 between suppressor cells and effector cells was required to induce suppression of the PFC response.

## DISCUSSION

The fetus, although antigenically foreign, is normally not rejected by its mother. Many hypotheses have been proposed to explain this biological and immunological paradox, but some of the older ideas such as the uterus being an immunologically privileged site, antigenic immaturity of the fetus or an anatomical fetomaternal separation may no longer be valid (for discussion see 16). To date, one of the most attractive theories which explain the successful fetomaternal transplantation is the local production of soluble immunosuppressive factors such as human chorionic gonadotropin (1, 5, 10, 11), human chorionic somatomammotropin (5) and alpha-feto-protein (24). Since the amounts of HCG adjacent to the trophoblasts exceed the concentrations needed for suppression of immune responses (4), these results argue in favor of the assumption that this hormone may constitute a pregnancy retaining factor. The difference in concentrations between retroplacental and peripheral blood would then be of importance in protecting the fetus from rejection, leaving the overall maternal immunological resistance unimpaired (8).

It is suggested that the mechanism by which alpha-feto-protein exerts its putative immunosuppressive effect is by the induction of suppressor T cells (17). Antigen induced suppressor cells, a distinct subpopulation of T cells, were described in the beginning of the 1970s by Gershon and coworkers (9, for review see 18) who showed that specific immunological unresponsiveness to sheep red blood cells in mice could be passively transferred with lymphocytes.

## Table IV Effect of various amounts of HCG activated cells for suppression

Activated cells (%)	IgM anti NNP PFC/cult addition during precultivation	
	HCG 2 000 IE/ml	0
225		1 200
600		900
1 050		1 400
1 025		1 800
1 150		1 150

Cells from female C3H/Tif mice were cultivated for 48 hours in the presence of 2 000 IE of HCG/ml. Thereafter, the cells were harvested and various amounts of preculivated cells were added to syngeneic fresh spleen cells to result in a final concentration of  $10^7$  cells/ml. The admixture was cultivated in the presence of 100 µg/ml LPS. Cultures were harvested on day 3 and tested for the number of anti-NNP plaque forming cells.

Mitogen induced suppressor cells on the other hand have been shown to inhibit not only specific T cell dependent immune responses (6) but also a polyclonal response as induced by B cell mitogens such as LPS and PPD (21-22). The results presented in this paper suggest that HCG induced cells may act in a similar fashion to those induced by T cell mitogens (concanavalin A). The effect was not due to cytotoxicity since cell survival was not affected nor was it due to passive transfer of HCG since freeze killed cells were nonsuppressive. The cells were found to resemble T cells as shown by their capacity to pass through a nylon wool column. This is in agreement with earlier findings showing that a majority of suppressor cells are of T cell origin (18). In this study only female mice were used mainly to imitate a natural biological model and also because human fetal cells have previously been shown to suppress a mitogen induced response of the maternal cells (19).

However the fetomaternal relationship may be guaranteed not only by means of soluble factors produced by the mother or fetus but also by a complex mechanism with different factors involved all contributing to the successful outcome of pregnancy. In this context it may be appropriate to make a direct comparison between pregnancy and some of the genetic factors known to be related to graft survival in surgically transplanted patients. In all species major histocompatibility complex encoded products presented on the cell surface are responsible for induction of the homograft reaction. In man these co-

dominantly expressed antigenic markers are determined by genes within one region on chromosome 6. This part of the genome is composed of different loci where the D locus seems to be the most important one for transplant rejection. Recent data indicate that the one year survival of kidney transplants in certain instances may approach 80 per cent when donor and recipient share one D locus and thus are mismatched for only one D allele (23). This may be comparable with the fetomaternal relationship where only half of the genetic material is antigenically foreign to the mother and since the frequency of early spontaneous abortions ranges between 15-30 per cent (3-15) the successful outcome of pregnancy in most cases may be due to a single D locus dissimilarity but other immunosuppressive factors may contribute (for discussion see 10). The biological relevance of studies in which it is shown that HCG is immunosuppressive can at this stage not be fully evaluated. In this study however we demonstrate that one possible mecha-

nism of HCG induced immunosuppression is the generation of suppressor T cells theoretically capable to impair transplantation antigen induced lymphocyte activation of maternal cells.

# ACKNOWLEDGEMENTS

This work was supported by the Swedish Cancer Society and the Torsten and Ragnar Söderberg Foundation. We gratefully acknowledge the skilful technical assistance of Ms. Caroline Malmgren.

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*Submitted for publication March 27 1979*

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## A NEW JOURNAL

**Journal of Foetal Medicine** Editor in Chief B. A. Salvadori Institute of Obstetrics and Gynaecology  
University of Parma Via Repubblica 58 43100 Parma Italy

### *Publication schedule and subscription information*

Annual subscription fee (4 issues) US dollar 50 Commencing publication mid 1990

*Publisher* SEMES s r l Ed. PADUA (Italy) *Responsible Manager* Prof. Antonio Ottolenghi

*This journal is intended for the publication of material within the field of Foetal Medicine regarded as a branch of medical science devoted to the study of the fetus and its environment under normal and pathological conditions. By propagating contribution from morphologists, immunologists, biochemists, geneticists, immunologists as well as the clinical and experimental data of obstetricians, pediatricians, the journal constitutes the first specific publication in its field.*

*The Journal of Foetal Medicine will accept original and high scientific quality contributions in the form of papers and short communications. Reviews and articles will be published following an invitation from the Editor in Chief or Associate Editors.*

## GRANULOSA CELL AND THECA CELL TUMORS

The clinical picture and long term outcome for the Radiumhemmet series

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Over the period 1923-72 a total of 305 patients with granulosa-cell, theca-cell tumor and mixed tumor were treated at Radiumhemmet. The most common symptom was abnormal uterine bleeding. In 13 per cent of married women were nulliparous. Menarche seems to have occurred earlier in this group than in the general population. The age at onset of the granulosa-cell tumor group gave birth to 12 children after treatment for the tumor. Fourteen women with granulosa-cell tumor and 4 with thecoma had received radiotherapy earlier in life for benign tumors. The risk of endometrial cancer was approximately 10 per cent greater for the women with granulosa- or theca-cell tumors than for the general population. One hundred and fifty-two patients were given both surgical treatment and radiotherapy. Fifty-three received surgery (37 granulosa-cell and 16 theca-cell tumors). One of the thecoma patients but 21 per cent of those with granulosa-cell tumor died from their disease. The 5-year survival for the latter group all stages was 85 per

cent — are almost invariably benign (16). Both granulosa and theca-cell tumors are often estrogenic and a distinctive clinical history of abnormal uterine bleeding is usual. The simultaneous occurrence of the two types of tumors and adenocarcinoma of the endometrium is frequently reported (7).

As a consequence of the centralized organization of cancer treatment in Sweden a relatively large number of women with granulosa- and theca-cell tumors have been treated at Radiumhemmet, Stockholm. The purpose of this study was to analyze this material from various clinical aspects and to examine the prognosis for the patient with either of these types of tumors.

## MATERIAL AND METHODS

Granulosa- and theca-cell tumors together constitute the largest group of functioning ovarian neoplasms. Granulosa-cell tumors occur as mixed neoplasms containing both granulosa and theca cells. For the 4-year period 1962-65 the annual incidence rate for granulosa- and theca-cell tumors in Sweden was 1.70 per 100 000 of the female population, thus the highest figure reported by any Western country (9). Granulosa-cell tumors and thecomas constituted 8 per cent of all ovarian neoplasms treated at Radiumhemmet (15). According to other studies between 5 and 10 per cent of all malignant ovarian tumors are of the granulosa-cell type (16). The malignant potential of this tumor has been variously reported. Novak gave a figure of 25 per cent. So far no unequivocal clinical or histologic method of the long term prognosis for the patient has been identified. Pure theca-cell tumors — thecomas

Between 1923 and 1972 a total of 263 women with histologically verified granulosa- or mixed granulosa- and theca-cell tumors (referred to below as granulosa-cell tumors) and 42 with pure thecomas were seen at Radiumhemmet. They had been referred from various parts of Sweden, one half of them from the Stockholm area. All the histological specimens were re-examined at the Department of Tumor Pathology at the time of admission. All the available endometrial specimens were also reviewed in connection with this study. A follow up of all the patients was performed in 1977-78. This was greatly facilitated by the system of civil registration and personal identification numbers used in Sweden. The population registry furnished data on the patients including date and cause of death and the name of the physician signing the death certificate, which information enabled the patient's subsequent hospital records and autopsy reports to be traced. Information from the population registry and the medical reports was punched on cards for statistical analysis. The clinical picture was analyzed for the two tumor groups. No histological grouping of the granulosa-cell tumors according to cell pattern was made. *Statistical methods* The survival rate was analyzed with life tables using a computer program. The expected survival of a population with the same age distribution as the granulosa-cell tumor series was calculated from life tables for the Swedish female population.

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Table III Radiotherapy given earlier in life to the granulosa-cell tumor and thecoma patients

	Granulosa-cell tumor patients		Thecoma patients	
	No	Interval between diagnosis and therapy year	No	Interval between diagnosis and therapy year
omen	0		1	14
st region	3	1 4 4	0	
d and neck	3	10 13 III	0	
ts etc	4	2 3 10 26	0	
acavitary	3	2 3 3	1	19
iration	6	1 2 4 5 5 24	2	III 26
il	19		4	

rhesis groups in 124 cases 87 per cent of whom were Rh positive. The distribution by blood group is given in Table II where a comparison is made with the Finnish population as a whole (8).

**Radiotherapy prior to diagnosis:** Nineteen women with granulosa-cell tumor and 4 with thecoma had received radiotherapy for benign lesions earlier in life. The site and the interval prior to diagnosis are presented in Table III.

**Other malignant diseases:** All malignant diseases were recorded either from the patients' hospital records or from death certificates. In the case of a new malignant disease in a patient once treated at Radiumhemmet Institution was notified a few malignant tumors were found at autopsy. Twenty per cent of the women had at least one such disease, the commonest being endometrial carcinoma with 33 cases of whom 10 could be reviewed and were confirmed. In 29 of these this tumor occurred simultaneously with the ovarian tumor (Table IV). Nine women had breast cancer (7 of the granulosa-cell tumor and 2 of the thecoma groups). A number of other malignant diseases were recorded in the remaining patients. All patients but one with endometrial carcinoma had reached the menopause; the exception was 47 years old and had a thecoma.

**Endometrial alterations:** Endometrial biopsy reports from the ovarian operation were available for over one half of the patients with granulosa-cell tumor and for three quarters of the thecoma group. Glandular cystic hyperplasia occurred in 45 per cent of the former group and 13 per cent of the latter.

**Clinical stage:** Surgical reports were available for all but one of the patients. The tumor was classified as Stage I (FIGO) in 90 per cent of the granulosa-cell tumor patients and in all the thecoma group. The tumors were bilateral in 2 and 5 per cent respectively.

**Tumor size:** Where the size of the tumor had been expressed by comparison to fruits, nuts, eggs and the like, it was converted into centimeters in diameter (Table V). While the granulosa-cell tumors varied greatly in size they tended to be larger than the thecomas, with 42 per cent at least 11 cm in diameter compared with 12 per cent of the thecomas.

**Treatment:** The treatment given to these patients has varied over the years. In 53 of the 305 patients surgery was the sole measure (37 with granulosa-cell tumor and 16 with thecoma); in the remainder radiotherapy was also given. The operative technique ranged from simple resection of the ovary or unilateral oophorectomy to radical surgery including omental resection. The radiotherapy consisted in intracavitary radium or external irradiation or a combination of these. Con-

Table IV Endometrial carcinoma occurring after or simultaneously with the ovarian tumor

	Endometrial carcinoma	
	Total	Co-existing with the ovarian tumor
Granulosa-cell tumor	21	10
Thecoma	12	10
	33	20

Table V Granulosa-cell tumors and thecomas distributed by size expressed as the diameter

Tumor diameter cm	Percentage of tumors							
	1	2	3	5	8	11	20	
Granulosa-cell tumors (257)	5	3	7	15	28	28	14	
Thecomas (47)	7	7	24	24	26	7	5	



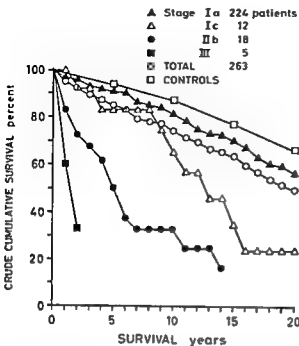


Fig. 2 Crude cumulative percentage of survivors for the various clinical stages of granulosa cell tumor compared with the expected survival calculated from life tables for the general Swedish female population

ventional X rays used in the earlier cases were superseded by high voltage techniques

**The follow up** At the follow up in 1977–78 nearly half of the women were living 60 per cent of them having a survival time of 15 years or more (range – 47 years) only one woman was lost to the study

In only a few patients was the cause of death verified by autopsy the entry in the report being based on the death certificate All deaths from granulosa cell tumor were verified either clinically or histologically

**Causes of death** Twenty-one per cent of the patients with granulosa cell tumor died of the disease and a further 4 per cent had a recurrence these figures serve as an indication of the neoplasm's malignant potential In the granulosa cell tumor group 9 per cent succumbed to other malignant diseases 3 of them to endometrial carcinoma (one of these being diagnosed at the same time as the ovarian tumor) and 5 to breast cancer

In none of the thecoma patients was this tumor the cause of death but 14 per cent died of other malignant diseases Two deaths were due to endometrial carcinoma (one of them being diagnosed at the same time as the ovarian neoplasm) cancer of the breast colon papilla Vateri and sarcoma of the uterus

claimed one patient each The most common cause of death other than malignant tumor were cancer and cardiovascular diseases

**Survival** The crude cumulative percentage of survivors for the various clinical stages of the granulosa cell tumor is presented in Fig. 2 As a reference expected survival curve for a population of the same age structure drawn on the basis of Swedish life tables is given The outcome was most favorable for Stage Ia (unilateral tumors) (FIGO) with 51 per cent of the patients surviving 5 years and 31 per cent 10 years The corresponding figures for Stage Ib were 50 and 32 per cent The expected figures for the general female population were 51 and 47 per cent respectively

## DISCUSSION

The findings of this study are consistent with the earlier ones as regards age distribution (3, 9, 12) abnormal uterine bleeding (3, 5) age at menopause (1) and the occurrence of breast cancer There was no evidence of a familial factor distribution by blood group did not differ from that of the general Swedish population (8)

Most of the patients in this series had reached menarche in the decade 1914–24 To judge from reference figures for the general population at the same time (17) the mean age at the menarche for the women in our series seems to have occurred somewhat earlier than the average

Of the premenopausal women with granulosa cell tumor 16 per cent had a history of secondary amenorrhea An annual incidence of 3.3 per cent has been reported in a randomly selected Swedish population in the age range 35–45 (14)

The number of nulliparous married women with granulosa cell tumor was three times greater than reported by Kolstad & Beecham (1975) and was also higher than the mean for Norwegian married women (9) The relationship between hormone secreting tumors of the ovary and sterility is difficult to quantify but a great majority of women with such tumors stop child bearing age It should be noted however that child bearing may be possible after treatment of granulosa and theca-cell tumors and that the tumor may sometimes be cured

In view of the evident connection between endometrial cancer and granulosa or theca-cell tumor endometrial biopsy specimens would be expected to contain information on precancerous changes

ometrium Glandular cystic hyperplasia of the endometrium was common among the patients in the granulosa cell tumor group but much less so in the theca cell group probably because its potential development is masked by the presence of endometrial carcinoma in many of these patients. From age adjusted expected incidence rates (Swedish Cancer Registry 1965) it would seem that the patient with a granulosa or a theca-cell tumor runs a ten fold greater risk of developing endometrial carcinoma. It is often argued that because of the difficulty in reaching a correct diagnosis in a patient with long hormone stimulation of the endometrium many of the diagnoses of endometrial carcinoma in patients with granulosa or theca-cell tumors may be incorrect. All but 3 of the observed 33 cases in the present series were reviewed and confirmed. 5 women died from the disease. Carcinomatous changes in other types of cancer are probably due to years of exposure to irritants or to substances inducing hyperestrogenia. In the case of endometrial cancer estrogen is most certainly involved. For a reliable evaluation of a causal relationship the types and amounts of estrogen produced by the tumor must be known. It is recognized that granulosa cell tumors can be induced in mice by irradiation (6). There are specific reports of women who had received radiotherapy and subsequently developing granulosa or theca-cell tumors (10). As many as 7 per cent of the present series of granulosa-cell tumor patients had at some time undergone radiotherapy. In one half of these (gynecological complaints) the radiotherapy may have been given for symptoms evoked by the yet undetected tumor. The survival rate for the granulosa-cell tumor patients was slightly worse than that for the general population. The stage of the tumor has proved to be an important prognostic factor. The fact that 21 per cent of the women with granulosa-cell tumor died from the disease is clear evidence of its malignant nature. Though a few malignant thecomas have been reported their existence is widely doubted (16). None of the theca-cell tumors in this series proved to be malignant.

#### ACKNOWLEDGEMENTS

This study was supported by the Swedish Cancer Society and the King Gustaf Vth Jubilee Fund. We thank Dr Claes Siltversward for reviewing the endometrial biopsy specimens.

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Submitted for publication January 16 1979

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## SHORT COMMUNICATION

RELIEF OF MENSTRUAL DISCOMFORT AND DYSMENORRHEA AND  
SIMULTANEOUS SUPPRESSION OF UTERINE ACTIVITY BY ISOXEPAC

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steroidal anti-inflammatory agents used for the treatment of dysmenorrhea have already been the subject of a special meeting (1) confirming their importance in modern science (3). Although it has not been possible to differentiate between the analgesic and specific effects of these agents on muscle spasm (2) it is nevertheless clear that such compounds decrease both premenstrually elevated prostaglandins and high uterine activity (4). Isoxepac,  $\alpha$ -dihydroxy-11-oxodibenz(b,e)-oxepin-2-acetic acid, is a new non-steroidal anti-inflammatory agent with a marked difference between the dose which gives anti-inflammatory properties and that which causes gastrointestinal irritation (2). Isoxepac might be suitable for the treatment of menstrual discomfort as well as primary dysmenorrhea.

A single-blind study was conducted on the first day of menstrual bleeding to determine the effect of isoxepac on menstrual discomfort in ten normal

gravida volunteers (age  $22 \pm 1$  years, mean  $\pm$  SE) and on dysmenorrheic pain in eight patients ( $30 \pm 3$  years, mean  $\pm$  SE). The patients were not taking oral contraceptives and no organic cause of the dysmenorrhea was found. In order to exclude patients with abnormal basic endocrinology, plasma progesterone and estradiol 17 $\beta$  were analyzed by RIA on cycle day 1. Intra-uterine pressure (IUP) was recorded for 210 min to study the effect of a prostaglandin synthetase inhibitor, isoxepac, on uterine activity in normal and dysmenorrheic patients. After a 30 min adjustment period the placebo effect was recorded for 1 hour and the isoxepac effect for 2 hours. Isoxepac was used as a single oral dose of 200 mg (7 cases) or 600 mg (11 cases). No side-effects were observed. Significant relief of pain occurred after isoxepac administration ( $p < 0.001$  (Table I)). The lower dose of 200 mg was as effective as the 600 mg dose. Ninety minutes after the administration of isoxepac only one case under psychiatric therapy was

still having moderate pain. Pains, if they returned, recurred on the average 6 hours after a single dose of isoxepac.

Analyses of intrauterine pressure during placebo and isoxepac treatment showed the same basic trend as that observed when other prostaglandin synthetase inhibitors were used (4).

One normal woman with a high active pressure (amplitude) but with a low frequency of cycles and with a low resting pressure (tone) experienced only menstrual discomfort (Fig. 1). After a single dose of 600 mg isoxepac active pressure decreased and the menstrual discomfort disappeared 30 min after its administration. The high active uterine pressure did not cause dysmenorrhea because the low resting pressure and low frequency of cycles guaranteed normal uterine blood flow (1, 4).

One normal woman had abnormally high plasma progesterone (5.5 ng/ml) and estradiol 17 $\beta$  (0.13 ng/ml) levels on the first day of bleeding. Uterine activity was negligible (Fig. 2) as compared with the average IUP found (Table II). This case was therefore not considered in later statistics. As shown in Fig. 3, in a dysmenorrheic woman isoxepac reduced all parameters of IUP: active pressure (AP), frequency of cycles (F) and resting pressure (RP). Average changes in IUP followed a similar course in normal and dysmenorrheic women (Table II).

The reduction of IUP by isoxepac was also statistically significant ( $p < 0.05$ ) in normal women when every patient was considered as her own control.

Isoxepac, a new non-steroidal anti-inflammatory agent, relieves menstrual discomfort, dysmenorrheic pain and reduces uterine activity during the menstrual period. It can be considered as a new alternative when the problems of menstrual discomfort and dysmenorrhea, a major social problem, must be faced by gynecologists.

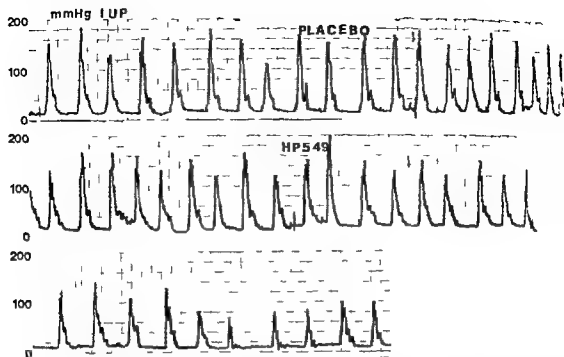


Fig 1 Note the high active pressure ( amplitude ) but low frequency of pressure cycles and low resting pressure ( tone ) This normal woman experienced only menstrual discomfort relieved by Isoxepac (HP 549) treatment scale minutes

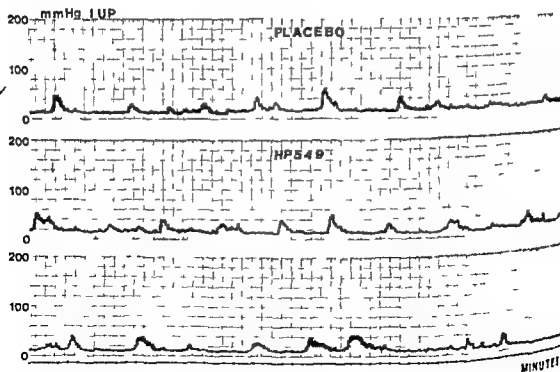
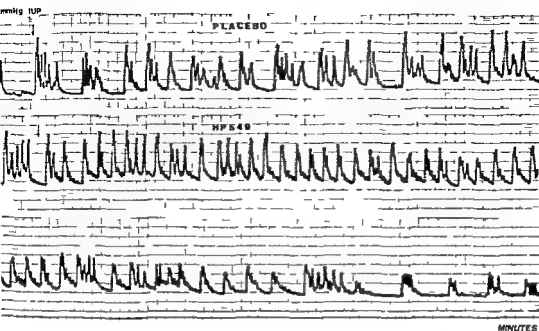


Fig 2 The very low uterine activity in this normal patient was found to be coincident with high plasma progesterone (5.5 ng/ml) and estradiol 17 $\beta$  (0.13 ng/ml) at the onset of bleeding



3 Isoxepac (HP 549) 600 mg oral reduced all parameters of intra uterine pressure in this dysmenorrheic woman relieved her pain

Table I Relief of menstrual discomfort or pain by isoxepac

	Before treatment	Placebo treatment	Isoxepac 1st hour	Treatment 2nd hour
Normals (10)	2.0 ± 0.7	1.9 ± 0.7	1.5 ± 0.7	1.1 ± 0.3
Dysmenorrheic (8)	3.4 ± 1.1	3.3 ± 1.2	2.8 ± 1.2	1.7 ± 1.1

0.001 Mean ± S.E. (n) Score 1 = no pain 2 = discomfort 3 = moderate 4 = severe 5 = very severe pain

Table II Intra uterine pressure in normal and dysmenorrheic women during isoxepac treatment

	Resting pressure mmHg	Active pressure mmHg	Frequency of pressure cycles in 30 min
Normals (9)			
Placebo	23 ± 3	63 ± 12	28 ± 6
Isoxepac 2nd hour	17 ± 2	51 ± 7	20 ± 4
Dysmenorrhea (8)			
Placebo	30 ± 3	59 ± 6	14 ± 1
Isoxepac 2nd hour	21 ± 5	44 ± 4	10 ± 1

0.05 Mean ± S.E. (n)

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*Submitted for publication November 23 1978*

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## CASE REPORT

## PERINATAL ASPHYXIA IN SPITE OF A NORMAL CARDIOTOCOGRAM AND A NORMAL ACID BASE STATE AT THE TIME OF DELIVERY

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**Abstract** The labor preceding delivery of a depressed male with normal acid base status is described. Cardiotocographic (CTG) monitoring was used during the 5.5 hours of active labor. Continuous tissue pH determination was performed during the last 100 minutes of labor. Although the CTG pattern was normal all the time, tissue pH declined to 7.07 one hour before delivery but reached normal values thereafter. The depression of the neonate could be explained by the episode of acidosis one hour before delivery.

Although most cases of asphyxia can be detected by cardiotocography, some newborns may be delivered with asphyxia and a low umbilical artery pH in spite of a normal cardiotocogram which has appeared normal during labor (4).

Furthermore, although the fetal pH at delivery corresponds very well to the Apgar score (3), a normal acid base state in the blood of the umbilical vessels can be found in depressed neonates (1). The depression may be caused by drugs, infections, airway obstruction, genital anomalies, precipitous delivery, prematurity, and also by a previous episode of asphyxia and a change of acid base state but not of the responsiveness of the central nervous system.

The following case illustrates that the last cause mentioned by James *et al.* for a lack of agreement between the Apgar score and the acid base state of the newborn is not only theoretical. The case also illustrates that the cardiotocographic pattern can be normal in spite of a decreasing tissue pH.

## CASE REPORT

A 36-year-old para 2 with a pregnancy which was normal until the 36th week of gestation was admitted to our department because of decreasing HPL (human placental lactogen) values but normal estron values. As the HPL values

remained low, labor was induced by artificial rupture of membranes (ARM). Two hours later the contractions were stimulated by oxytocin (4 to 12 mU/min during the first stage of labor and 16 to 20 mU/min during the second stage of labor, total amount of oxytocin 5 Units). At the same time cardiotocography was initiated using an internal spiral ECG electrode and external registration of the contractions. Five hours after ARM, epidural analgesia was started using an epidural catheter because of painful contractions. No analgesics or other drugs than oxytocin were used. An hour later (6 hours after ARM) the cervix was dilated 5 cm and a pH electrode was applied together with an intrauterine catheter (2). The course of the rest of labor was uneventful, the second stage only lasting 10 minutes. The total duration of labor (from ARM) was 7.5 hours.

The CTG was normal before induction of labor, during the beginning of induction, and throughout the first stage of labor. Figs 1-3 show a normal CTG with isolated accelerations simultaneous with the contractions, a normal base line and variability, and very few early decelerations, especially during the last 30 minutes of the first stage. During the second stage, which lasted only 10 minutes, the baseline declined from 140 to 120 beats per minute and the early decelerations became more pronounced, but the fetal heart rate reached the baseline level again immediately after the contractions.

The tissue pH was registered for the first time 100 minutes prior to delivery (Fig. 1). Fig. 1 shows the decrease of pH from 7.22 to 7.02 during the first 30 minutes of pH monitoring. As this decline was thought to be a false low pH reading (CTG normal) and as a low tissue pH in itself at this stage of the investigation is not an indication for obstetrical intervention, there was no interference. During the next hour (Figs. 1 and 2) the pH increased, reaching a maximum level of 7.11 (Fig. 2). During the second stage (Fig. 3) the pH decreased from 7.11 to 7.21, the last value corresponding very well to the umbilical artery pH of 7.24 (umbilical vein pH 7.31). The other acid base values and the oxygenation of the umbilical artery were also normal (base excess -5 mmol/l,  $pCO_2$  56 mm Hg, standard bicarbonate 17.8 mmol/l,  $pO_2$  9 mm Hg, oxygen saturation 16 per cent) when measured on autoanalyzers (ABL 2 and OSM 1, both Radiometer Copenhagen).

A cyanotic, hypotonic infant was delivered. Apgar scores were 2, 8 and 10 respectively one, three and five minutes



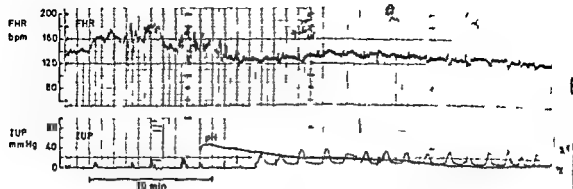


Fig 1 Cardiotocogram (CTG) including tissue pH values 75–115 minutes before delivery. The CTG showed a normal baseline level and variability and a few early decelerations (maximum 15 beats per minute). The acceleration

before tissue pH registration is caused by the movement of the pH electrode. The pH was 7.23 at the time of registration. 25 minutes later the pH had decreased to 7.12. At the end of the period the pH was 7.05.

after delivery. Birthweight 2900 g, length 51 cm. The infant was admitted to the intensive neonatal department because of respiratory distress and perinatal asphyxia. Oxygen and CPAP (Continuous Positive Airway Pressure) treatment was necessary until the infant was three days old. On the seventh day the child was discharged with a normal neurological status. No infections, anomalies or other explanations for the perinatal depression were found.

### DISCUSSION

The case illustrates that intrauterine acidosis can develop without the CTG being abnormal. The very of a depressed but otherwise healthy infant with a normal acid base state could not be explained by drug administration during labor. The episode of

acidosis one hour before delivery could very well explain the neonatal depression, as it has been reported that the central nervous system is depressed for a certain period after normalizing the acid base equilibrium (1).

If more cases similar to the one registered in the future, this would be strong evidence of the need for tissue pH monitoring in addition to monitored with cardiotocography. As cardiotocography cannot always reveal asphyxiation of the fetus, this however requires a better pH monitoring system in particular one which does not give false pH values, because the risk of such false pH values makes it impossible to intervene when a pH reading is the only sign of fetal distress.

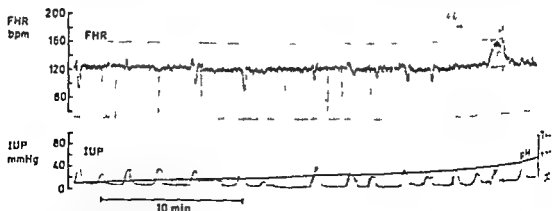


Fig 2 Cardiotocogram (CTG) including tissue pH values 40–75 minutes before delivery. The CTG showed a normal baseline and variability and a few early decelerations (max

imum 25 beats per minute). The acceleration was seen at a vaginal examination. The tissue pH increased to 7.20.

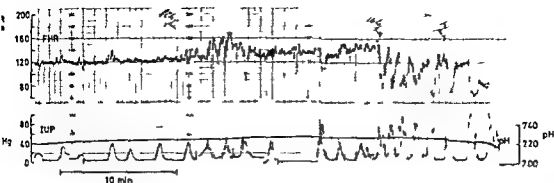


Fig. 3. Cardiotocogram (CTG) including tissue pH values 10 minutes before delivery (the last 10 minutes represent second stage). First stage: normal baseline level and variability and a few early decelerations (maximum 50 beats per minute). Second stage: the baseline decreased from 140

to 120 beats per minute, normal variability and early decelerations (maximum 60 beats per minute). The tissue pH reached a maximum of 7.28 at the beginning of the second stage, thereafter decreasing to 7.22 during the rest of the second stage. The umbilical artery pH was 7.24.

### ACKNOWLEDGEMENT

Work was supported by the Dagmar Marshall Fund.

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Submitted for publication December 15, 1979.

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## CASE REPORT

GAUCHER'S DISEASE IN PREGNANCY ASSOCIATED WITH  
INTRA UTERINE GROWTH RETARDATION

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Gaucher's disease associated with pregnancy is rare. 33 cases have been reported (3).

The disease was originally described by Gaucher in 1876 and he thought it was caused by a primary epithelioma. It is now known that the disease is an inborn error of intra-cellular metabolism characterized by abnormal storage of cerebroside in theiculo-endothelial cells (1).

Originally it was believed that therapeutic abortion was indicated when pregnancy was superimposed on Gaucher's disease. This has since been refuted. There is no significant increase in the number of hemorrhagic complications of pregnancy, labor or puerperium have been found.

We present a patient first diagnosed as having Gaucher's disease in pregnancy because she developed hemorrhagic complications and a progressive decrease in the growth rate of the fetus. Spontaneous improvement in the platelet count which occurred from 33 to 38 weeks coincided with improved foetal growth.

The possible association between Gaucher's disease and its hemorrhagic complications and intra uterine growth retardation is therefore suggested.

## CASE REPORT

A patient aged 30 years was of Jewish South African origin and was 22 weeks pregnant in her first pregnancy. She was admitted with vaginal bleeding. She gave a history of recent onset of rectal bleeding and bleeding from gums. On examination her weight was 69.8 kg, she was not jaundiced, there was a conjunctival haemorrhage of the right eye, peri-orbital bruising as well as marked bruising of the knee. The uterus was the correct size for dates and the foetus was easily palpable. Her haemoglobin was 11.8 g/dl, white cell count 8.4 x 10<sup>9</sup>/l, platelets 100 x 10<sup>9</sup>/l. The clotting factors and blood urea were normal.

Her father was known to have an aplastic anaemia of one year's duration and her sister had been diagnosed as having Gaucher's disease.

A bone marrow biopsy was performed which showed typical Gaucher cells.

The vaginal bleeding stopped, no further bruising occurred and she was therefore discharged. Her platelet count became 125 x 10<sup>9</sup>/l.

She was readmitted when 29 weeks pregnant with further bruising, the platelet count being 95 x 10<sup>9</sup>/l. Her weight was 69.5 kg and clinically the uterus was small for dates.

Her weight continued to fall and at 33 weeks she reached her lowest weight of 68.2 kg. Her platelet count also fell progressively to 70 x 10<sup>9</sup>/l.

From 33 to 38 weeks she gained weight slightly, her weight reaching 70.8 kg. Over the same period her platelet count improved spontaneously rising to 130 x 10<sup>9</sup>/l. Serial ultrasound biparietal diameter measurements showed an improved growth rate from 33 to 38 weeks.

At 38 weeks a lower segment Caesarean section was performed. A live healthy male infant weighing 2.01 kg was delivered. The placenta weighed 440 g and blood loss at Caesarean section was 700 ml. The post-operative course was uncomplicated. She was discharged on the 12th post-operative day, her platelet count being 175 x 10<sup>9</sup>/l. She weighed 64.2 kg which had been her non-pregnant weight. The baby developed no neonatal complications.

## COMMENT

Previous authors (1-3) have concluded that in cases of Gaucher's disease in pregnancy, if close hematological investigation is undertaken, a normal antenatal course can be expected with a good fetal outcome.

Our patient is extremely interesting in that she presented during pregnancy for the first time with hemorrhagic complications and indeed this led to the diagnosis of Gaucher's disease first being made. In addition the growth rate of the fetus decreased markedly from 28 to 33 weeks which coincided with a progressive fall in platelet levels. Improvement in the fetal growth rate also coincided with a spontaneous

increase in the platelet count from 33 to 38 weeks. It is also of interest that her total weight gain in pregnancy was only 6.5 kg.

# ACKNOWLEDGEMENTS

We wish to thank Mr P Chalk for allowing us to present this case and Miss L Epsztejn for her assistance in preparing this paper.

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*Submitted for publication November 20 1979*

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## CASE REPORT

## A MINOR COMPLICATION OF CRYOSURGERY — OCCLUSION OF THE CERVICAL CANAL

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**Abstract** In a series of 145 patients who underwent surgery of the cervix uteri six cases (4 per cent) were identified with occlusion of the cervical canal. The patients complained of constant pains resembling labor. Inspection of the cervix uteri showed necrosis covering the os internum uteri. On removal of the necrotic area there was a large amount of watery fluid. The patients were then free of

surgery was performed in two sessions there was a thawing period of four minutes between sessions.

After completion of the cryosurgery the ice ball on the cervix thawed in the course of five to fifteen minutes. At that stage the macroscopic appearance of the portio was unchanged from its appearance before treatment. During the first twenty-four hours after cryosurgery patients experienced a profuse watery discharge which continued for two to six weeks. After one to three days there was necrosis of the superficial part of the portio that had undergone cryosurgery. The necrotic tissue sloughed spontaneously after one to two weeks. The portio healed completely during the next few weeks.

During the last decade cryosurgery has been increasingly used in the treatment of benign and premalignant conditions of the vulva, vagina and cervix uteri. Treatment usually involves no discomfort for the patient. It is quick, easy and painless and may therefore be performed on an outpatient basis. Cryosurgery gives satisfactory results and suffers from a few complications; its application will probably become even more widespread.

What follows we will describe one of the minor complications which we have called the "plug syndrome" and which has been described in the literature only once before. This syndrome has occurred in 4 per cent of our 145 patients.

## CASE REPORTS

**Case 1** A 30-year-old woman with moderate dysplasia. Cryosurgery was performed in two sessions of 15 seconds interrupted by a period of spontaneous thawing lasting four minutes.

The patient presented on the third day after treatment complaining of powerful pains resembling labor. There had been no vaginal bleeding but as expected there was a profuse watery discharge.

Inspection of the cervix showed a large area of necrosis covering the entire central part of the portio including the os ostium. Vaginal exploration showed normal condition in the pelvis. The necrotic tissue was removed without difficulty revealing the os ostium. There was an immediate escape of watery fluid mixed with a small amount of dark blood. The pains disappeared following removal of the necrosis and the patient remained pain free.

Menstruation like bleeding occurred for a few days corresponding to the expected time of menstruation. The patient subsequently experienced a watery discharge for three weeks.

**Case 2** A 28-year-old woman with mild dysplasia. Cryosurgery was performed in one session lasting 1.0 seconds.

The patient presented on the third day after treatment complaining of pains resembling labor. There had been no vaginal bleeding but there was the usual watery discharge.

Inspection of the collum showed a large area of necrosis covering the os ostium. Vaginal exploration showed normal conditions in the pelvis. The necrotic tissue was removed easily and a small amount of watery fluid escaped. The pains then disappeared.

## MATERIAL AND METHODS

The material consists of 145 patients who underwent cryosurgery of the cervix uteri for slight to moderate dysplasia. If these patients (4 per cent) developed symptoms corresponding to the "plug syndrome".

At the time of treatment none of the patients suffered from any gynecological complaints and menstruation was normal in all. There were no signs of salpingitis, vaginitis or infections. Cryosurgery was performed at an arbitrary time in the patient's menstrual cycle but the treatment was postponed if there was a possibility of pregnancy.

Cryosurgery was done with a cryopistol (Spembley 11R) powered with nitrous oxide ( $N_2O$ ). The patients were randomized to either one or two sessions of cryosurgery of one to three minutes per session and the aim was to create a frozen zone which extended at least three centimeters beyond pathological areas. When the cryo-

There was a normal menstrual period at the expected time three weeks after treatment. A watery discharge continued for three weeks.

**Case 3** A 35 year old woman with mild dysplasia. Cryosurgery was performed in two periods of 120 seconds each, separated by a period of spontaneous shawing lasting four minutes.

The patient presented on the fourth day after treatment with complaints of indisposition, abdominal pain and malarious discharge.

Inspection of the collum showed a large area of necrosis covering the ostium. Vaginal exploration showed normal conditions in the pelvis. The pain disappeared on removal of the necrotic tissue. After vaginal douching with a solution of chloramine for a few days the offensive odour disappeared.

There was a normal menstrual period at the expected time ten days after treatment. The watery discharge persisted for five weeks.

**Case 4** A 23 year-old woman with mild dysplasia. Cryosurgery was performed in one session lasting 130 seconds.

The patient presented on the sixth day after treatment complaining of pains resembling labor which had developed immediately after cryosurgery. There had been no vaginal bleeding but there was a profuse watery discharge.

Inspection of the cervix showed a large area of necrosis. Vaginal exploration showed normal conditions in the pelvis. The pains disappeared on removal of the necrotic tissue.

There was a normal menstrual period at the expected time 15 days after cryosurgery. The watery discharge continued for three weeks.

**Case 5** A 55 year-old woman with mild dysplasia three years after the menopause. Cryosurgery was performed in one session of 90 seconds.

The patient did not present herself in the department again until the agreed time of clinical follow up which was eight weeks after treatment. Here she stated that she had been troubled by severe pains resembling labor during the first two weeks after treatment. The pains disappeared spontaneously. There had been no periods of bleeding or fever. At the follow up examination the patient was symptomfree and the physical examination showed normal conditions. The watery discharge lasted for two weeks.

**Case 6** A 34 year-old woman with mild dysplasia. Cryosurgery was performed for a period of 105 seconds.

The day after treatment the patient was admitted to hospital with the diagnosis acute abdomen with pronounced abdominal pain, no vaginal bleeding but watery discharge. Her general condition was unaffected and her temperature was normal. Inspection of the cervix showed an approximately one centimeter thick translucent necrotic area covering the ostium. Vaginal exploration showed that the uterus was normal in size, mobile and moderately tender. There were no mass or tenderness at the sides. On admission the patient was put on antibiotic treatment for suspected commencing inflammatory adnexal disease.

The gynecological examination on the eighth day showed that the necrotic area had sloughed and that the peritum was healing with a well-defined ostium. There were now no further complaints about pain.

At no time during hospitalization was there any sign of inflammation; the temperature was normal and other or-

gan functions were found to be normal. There was a normal menstrual period at the expected time 11 days after treatment. The watery discharge lasted for 10 days.

## DISCUSSION

All six patients experienced pain from the first day to one to three days after the cryosurgery. The general condition of the patients remained unaffected; their temperature was normal. There was a watery discharge as in the case of other patients after cryosurgery. In four patients a slight tug was sufficient to remove the necrotic area from the ostium in one piece. Immediately after its removal there was a watery discharge from the uterus and pain disappeared. In patients Nos 5 and 6 the necrotic area sloughed spontaneously after which pain disappeared. In patient No 3 no occlusion of the cervix was observed but the case has nevertheless been excluded on account of the typical symptoms.

In these patients the characteristic feature was pain which developed when the ostium became occluded. As soon as a passage was established through the cervical canal by removal or sloughing of the necrotic plug the pain disappeared immediately. There can hardly be any doubt that the pain described, which we have called the "plug syndrome" is due to acute hematometra. The condition develops because the necrotic plug occluded by cryosurgery can obstruct the cervical canal. This complication is not serious as the pain disappears in the course of one to two weeks.

Galt (3) in 1975 described a single case of acute hematometra following cryosurgery. The patient presented the day after treatment with severe abdominal pains. A probe was passed through the cervical canal and about 15 ml of blood escaped after which the patient was symptomfree. This is the only case mentioned in the literature.

Creasman et al (1) have reported 12 cases of acute hematometra following cryosurgery in a series of 100 patients. Two of these patients presented with severe abdominal pain of the first week after treatment. The patients were affected by abdominal pains. The patients were affected by gynecological examination showed tenderness of the adnexa for which the patients received antibiotic treatment. The appearance of the cervix was normal. The course was otherwise unremarkable. These two cases could be excluded from the "plug syndrome".

is conceivable that the development of the plug syndrome might depend on the stage of the menstrual cycle when the cryosurgery was performed. Roth (2) finds it important to perform cryosurgery during the proliferative phase of the cycle in order to avoid hematometra. There were no complications in our series of 59 patients.

When carrying out cryosurgery we have not taken the time of the menstrual cycle into account. In the six patients with plug syndrome the relationship between the time of cryosurgery and the menstrual cycle was as follows: one patient had had her menopause 10 years previously, one patient had just ceased menstruating when the cryosurgery was performed, one underwent cryosurgery around the time of ovulation (two preovulation date, one postovulation date), and one underwent premenstrual cryosurgery. As for the other 139 patients in our series also underwent cryosurgery at a random time in the cycle, it may be assumed that this took place during the postovulatory phase in about half of the patients without giving rise to any problems. We feel justified in concluding that the development of the plug syndrome is independent of the time at which cryosurgery takes place in the menstrual cycle.

Even though it does not represent a serious complication since the patients will recover spontaneously during the course of one to two weeks, we have nevertheless considered it important to draw attention to the syndrome, as these patients can very easily be helped to avoid pain, unnecessary treatment with antibiotics, and in some cases unnecessary hospitalization.

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*Submitted for publication November 15, 1979*

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# CASE REPORT

## TORSION OF THE INTERNAL FEMALE GENITAL APPARATUS

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**Abstract** A case of a huge right-sided ovarian cyst associated with torsion of the whole internal female genital apparatus around the uterine collum, herniation of the transverse colon into the thoracic cavity and mechanical obstruction of the ileum is described. The symptomatology, pathogenesis and treatment of the condition are discussed.

We therefore consider it worth while presenting the following case of torsion of the uterus, fallopian tubes and both ovaries around the uterine collum complicated by diaphragmatic herniation of the transverse colon through Morgagni's foramen.

Torsion of pediculated ovarian cysts, fallopian tubes, uterine ovaries, adnexa and sacrosalpinges are well described disorders (1, 2, 4, 5). Pulmonary complications of large ovarian cysts have also been reported in the literature (3). However, common textbooks do not describe torsion of the whole internal female genital apparatus as a complication of ovarian cysts, and no specific information is available on this condition. Further, herniation of the colon through Morgagni's foramen has not previously been described as a complication.

# CASE REPORT

The patient was a 77-year-old female admitted because of intermittent abdominal colic localized to both hypochondric regions. Apart from operation for a femoral hernia at the age of 56 years, she had previously been in good health.

During the past two years she had noticed a gradual swelling of the abdomen, and in the last 5 months before admission she had had intermittent colic in both flanks and episodes of constipation. An intravenous pyelogram performed 4 months before admission was normal.

Because of increasing colic she was admitted as an emergency.



Fig. 1. Chest radiographs in the postero-anterior (a) and lateral (b) projections demonstrating herniation of the transverse colon into the right hemithorax.



Fig 2 Section of colon radiograph demonstrating a hernial ring at the site of Morgagni's foramen

Inspection and palpation of the abdomen revealed a fluctuating mass extending from the symphysis pubis to the xiphoid. The patient had slight orthopnoea but was in good general condition. Pelvic examination revealed a medium sized rectocele and a small atrophic uterine portio. The uterus could not be separated from the abdominal mass on

bimanual palpation. The preoperative work-up included an electrocardiogram which was normal, chest radiological examination of the colon and chest. The chest X-ray showed a large part of it located above the diaphragm in the right hemithorax (Fig 1a and 1b). The colon radiogram showed a hernial ring on the transverse colon localized in Morgagni's foramen in the right hemithorax (Fig 2).

The patient developed increasing symptoms of intestinal obstruction with nausea and vomiting. Emergency laparotomy was performed.

At operation a huge cyanotic right-sided mass occupying most of the abdominal cavity was found. The mass was emptied of 10 liters of brownish fluid, brownish pus. The huge right-sided ovarian cyst was excised, and the left adnexa had been torqued. The lower portion of a long atrophic column with hemorrhagic infarction of the whole internal genital apparatus. A well-defined line of demarcation between vital and necrotic tissue on the uterus. The uterine and cardinal ligaments were atrophic. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. The specimen is shown in Fig 3.

The transverse colon was easily pulled back into the abdomen along with the hernial sack which was resected after which a 5 x 3 cm large defect in the diaphragm at the site of Morgagni's foramen was closed (Fig 4). A portion of the ileum a conglomerate of mucus separated dilated from collapsed ileum. The abdominal cavity was divided upon which the intestines were resected.

Microscopy of the removed genital apparatus showed hemorrhagic necrosis of a multilocular right-sided cyst, the uterus and proximal colliculus, the fallopian tube and the left ovary. The amount of necrosis was extensive. The amount of necrosis was extensive. The amount of necrosis was extensive.

The postoperative course was unremarkable, and the wound infection was treated with antibiotics.

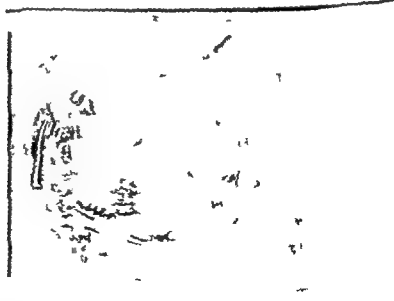


Fig 3 Specimen removed at abdominal hysterectomy with bilateral salpingo-oophorectomy. Note the large right-sided ovarian cyst (emptied), the necrosis of the whole internal genital apparatus and the line demarcating vital from necrotic tissue in the uterine colliculus.

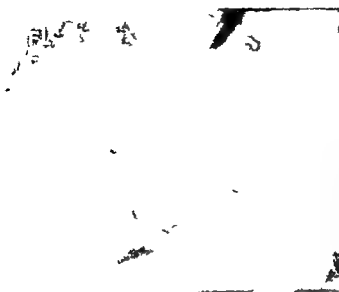


Fig 4 Peroperative picture demonstrating the hernial ring of the enlarged Morgagni's foramen L Liver FL Falciform ligament of the liver P Pericardium seen from the abdomen through Morgagni's foramen

## DISCUSSION

Torsion of the internal female genital apparatus is an extremely rare condition. The symptomatology of this syndrome can probably not be distinguished from that of simple torsion of a pediculated ovarian cyst. In the present case the size of the cyst had led to displacement of the transverse colon into the thoracic cavity through Morgagni's foramen. However the patient's main symptoms were caused by the mechanical obstruction of the ileum due to the adhesion.

We ascribe the pathogenesis of the condition to be primary torsion of the large right-sided ovarian

The momentum of this torsion on the uterine column along with the weak sacro-uterine and cardinal ligaments responsible for the fixation of the column of the uterus had eventually led to torsion of the whole internal apparatus.

Treatment of the condition is total or subtotal hysterectomy with bilateral salpingo-oophorectomy as soon as the diagnosis has been established since ischaemic necrosis of the genital apparatus with subsequent risk of infection may rapidly prove fatal.

## ACKNOWLEDGEMENT

We thank Kirsten Folke M B Head of the Department of Radiology for kind permission to use the radiographs.

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## PREMATURE RUPTURE OF THE MEMBRANES

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**Abstract** The etiology of premature rupture of the membranes (PROM) has been investigated in 30 consecutive cases and 30 matched controls. The significance of several possible factors predisposing to this condition is discussed and the obstetric and pediatric outcome reviewed. Significantly increased frequencies of previous genital operations, cervical lacerations and lacerations were found in the PROM group. The PROM group also contained significantly more heavy smokers. The difference between the length of the PROM delivery time and the risks of prematurity and infection are discussed. Patients delivered > 24 hours after PROM had significantly more puerperal infections than those with a latent period < 24 hours. Maternal fever was found to be an unreliable prognostic indicator. The incidence of puerperal infection amounted to 27 per cent in the PROM group. A 12 per cent incidence of proven neonatal septicaemia contributed to a high perinatal mortality rate (17.6 per cent).

In previous studies have attempted to elucidate the pathogenesis of premature rupture of the membranes (PROM) but so far no sole causative factor has been found. Among the explanations suggested mechanical factors including poor support such as a traumatic cervical incompetence combined with increased intra uterine pressure. Primary or later acquired defects of the fetal membranes have also been assumed to be causes of PROM.

The purpose of the present study was to reveal etiologic factors with a possible causative relation to PROM. Since infection following PROM is a real threat to mother and child the project also included an investigation of the microbiological flora of the genital tract in these patients with the underlying assumption that a PROM related infection is not only a complication but might possibly be of great significance. The hypothesis that as a consequence of infection the membranes may become weakened and subject to premature rupture has earlier been suggested by Knox & Hoerner (17) and Blake (5).

PROM is defined as spontaneous rupture of the amniochorionic membrane prior to the onset of uterine contractions. In this paper we report on the clinical findings, the microbiological results are published elsewhere (12).

## MATERIAL AND METHODS

**Patients**

**Workers.** All patients with PROM before the 36th week of gestation, 30 in all, admitted to the Department of Obstetrics and Gynecology during the period July 1976 to October 1977 have been included in the study. Every patient was matched with a control as regards age, parity, duration of pregnancy and socioeconomic status (all middle class).

The age distribution of the patients is shown in Fig. 1. PROM was diagnosed by means of vaginal examination which was sometimes confirmed by cytological examination for fetal cells, using the Nile blue sulphate staining procedure described by Litzch (16). Antenatal birth weight was es-

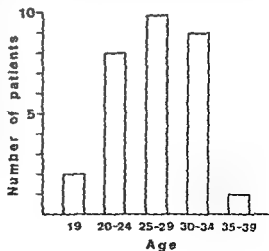


Fig. 1 Age distribution of patients

Table I Previous genital operations

	PROM	Controls
Exscesis after spontaneous abortion	4	6
Legal abortion suction procedure	12	5
Legal abortion 1 step procedure	2	2
Legal abortion abdominal hysterotomy	1	0
Dilatation and curettage	10	1
Conization	4	0
Cervical rupture repair	2	0
Laparoscopy	1	2
Extirpation of ovarian cyst	2	0
Salpingectomy	1	0
Extirpation of condylomas	3	0
Total	42	16

limited by clinical examination and measurement of the biparietal diameter by means of ultrasound. For the ultrasound measurements a Br  l and Kjaer real time equipment with a revolving sector scan transducer of 2 MHz was used and the calculations were made using the methods described by Campbell & Newman (9) and Thompson *et al* (30).

**Infants** Thirty three liveborn babies were admitted to the neonatal ward either immediately after birth or during the first week afterwards. Their medical histories were reviewed in retrospect. All infants were preterm i.e. 36 weeks of gestational age or less as assessed by maternal history, obstetric data and clinical examination.

The diagnosis of sepsis was based on clinical signs and a positive blood culture.

Chest X ray was performed on all babies with respiratory problems. Fulfilment of the following criteria was required to establish a diagnosis of idiopathic respiratory distress syndrome (IRDS): tachypnoea, retractions, grunting, increasing right to left shunt (i.e. oxygen requirement during the first 24 h) and typical radiological picture. Respiratory distress without increasing right to left shunt and not caused by other specific conditions is referred to here as transient tachypnoea.

Birth weight and size were compared with those given in Swedish neonatal growth charts (29).

Perinatal mortality is defined as the total number of infants who died *in utero* after 28 weeks of gestation or during their first week of life irrespective of gestational age. Autopsy was performed on all deceased infants.

**Statistical calculations** If not otherwise stated the calculations were made in the following way: fourfold tables were constructed on a pair basis for differences in qualitative

Table III Complications during the pregnancy

	PROM	Controls
Bleeding 1st trimester	9	1
Bleeding 3rd trimester	3	1
Infections	5	2
Toxaemia of pregnancy	0	1
Polyhydramnios	1	1
Premature contractions	6	1
No complications	10/30	17/16

variables and the hypothesis equal or less was used in the control group. was tested against the hypothesis higher frequencies in the observed group using the binomial (McNemar's) test (1).

## RESULTS

### Mothers

**Frequency** During the period 3391 parturients delivered in the Department. Thirty seven PROM were admitted corresponding to a frequency of 0.9 per cent.

**Previous gynaecological history** Forty per cent (primary or secondary involuntary sterility for years) preceding the last pregnancy were found. 23 per cent of the PROM patients compared with 7 per cent among the controls. The difference was not significant statistically, however. All previous genital infections were not found to be represented among the PROM patients.

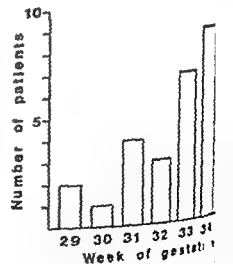


Fig 2 Time incidence of premature rupture of membranes

Table II Complications in previous pregnancies

	PROM	Controls
Bleeding	0	2
Toxaemia of pregnancy	5	2
Premature contractions	2	3
PROM	4	1
No complications	6/19	13/19

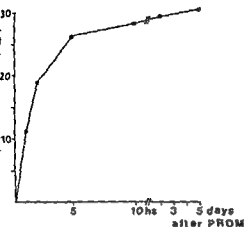


Fig. 3 Interval between premature rupture of the membranes and admission to hospital

differences in coital frequency in the two groups. In the case only did PROM follow directly upon

The distribution of previous genital operations is shown in Table I. These operations were found to be significantly overrepresented ( $p=0.01$ ) in the PROM group, the number of patients with more than one operation being significantly increased ( $p=0.05$ ). As could be expected there was an increased frequency ( $p=0.02$ ) of more obvious cervical trauma such as conization and cervical lacerations in the PROM material.

**Obstetric history.** There was no overrepresentation of multiparous women in the material, 40 per cent of the patients being nulliparas or primiparas. The PROM patients had no increased history of previous spontaneous abortions when compared with the controls. Legal abortions on the other hand were found to be more frequent among the PROM patients. A 15/7 ratio does not however correspond to statistical significance (Table I). The early versus late legal abortion did not differ significantly in the two groups.

Complications during previous pregnancies are listed in Table II. There was a higher incidence of toxæmia and PROM in the PROM group, though not statistically significant.

**Present pregnancy.** Complications during the present pregnancy are shown in Table III. There was a higher incidence of infections, premature contractions and bleeding in the PROM pregnancies, the last mentioned finding being the only one statistically significant ( $p=0.01$ ).

One case of acute enteritis preceding PROM was reported. This was the only case of intercurrent disease with a possible direct relation to the PROM.

Hard physical labor as a predisposing factor to PROM could not be verified. 4 patients in each group stating that they had arduous occupations.

The PROM group contained significantly more heavy smokers ( $>11$  cigarettes/day) than the control group ( $p=0.01$ ).

**Obstetric outcome.** The time incidence of PROM ranged from 29 to 35 weeks (Fig. 2). Two of the controls were delivered in the 36th week of gestation in both cases, however, without any signs of PROM, none of the other controls were preterm.

The time interval between the PROM and admission to hospital is shown in Fig. 3. The last 2 patients admitted arrived 2 and 5 days respectively after the PROM.

In the PROM group there were 4 twin pregnancies and 26 singletons. Among the latter there were 22 vertex and 4 breech presentations (15 per cent). The expected breech frequency of about 3 per cent at term was found among the controls. Twenty-five patients were delivered vaginally and 5 by Caesarean section (16.7 per cent). The average frequency of Caesarean section in the Department during the same period was 8.7 per cent. Altogether operative intervention was necessary in 8 cases: due to imminent asphyxia (3), infection (2), premature breech presentation (2) and transverse lie (1). The operations were Caesarean section (5), vacuum extraction (1) and manual extraction (2). Fifty-eight per cent of the patients primarily intended for conservative treatment subsequently needed operative intervention, as compared with 5 per cent among the actively treated subjects. The difference reflects the fact that the latter group contained the more mature infants. Of the 4 twin mothers 3 were delivered by Caesarean section and the fourth was delivered vaginally because of infection. Three of the control patients (10 per cent) had a Caesarean section (indications: *feto-pelvic disproportion*, *partial abruption of the placenta*, *stillborn infant* + *sterilization*) while the rest were delivered vaginally without complications.

All placentas were sent for histopathological analysis. The extent of calcification and infarction was about the same in the two groups, amounting to about 15 per cent. Three of the PROM patients (10 per cent) had a placenta with abnormal umbilical cord insertion: one membranous and two marginal insertions. No such anatomical variations were found



Table I Previous genital operations

	PROM Controls	
Exarexis after spontaneous abortion	4	6
Legal abortion suction procedure	12	5
Legal abortion 2 step procedure	2	2
Legal abortion abdominal hysterotomy	1	0
Dilatation and curettage	10	1
Conization	4	0
Cervical rupture repair	2	0
Laparoscopy	1	2
Extirpation of ovarian cyst	2	0
Salpingectomy	1	0
Extirpation of condylomas	3	0
Total	42	16

timated by clinical examination and measurement of the biparietal diameter by means of ultrasound. For the ultrasound measurements a Brüel and Kjær real time equipment with a revolving sector scan transducer of 2 MHz was used and the calculations were made using the methods described by Campbell & Newman (9) and Thompson *et al* (30).

**Infants.** Thirty three liveborn babies were admitted to the neonatal ward either immediately after birth or during the first week afterwards. Their medical histories were reviewed in retrospect. All infants were preterm >= 36 weeks of gestational age or less as assessed by maternal history, obstetric data and clinical examination.

The diagnosis of sepsis was based on clinical signs and a positive blood culture.

Chest X-ray was performed on all babies with respiratory problems. Fulfilment of the following criteria was required to establish a diagnosis of idiopathic respiratory distress syndrome (IRDS): tachypnoea, retractions, grunting, increasing right to left shunt, i.e. oxygen requirement during the first 24 h, and typical radiological picture. Respiratory distress without increasing right to left shunt and not caused by other specific conditions is referred to here as transient tachypnoea.

Birth weight and size were compared with those given in Swedish neonatal growth charts (29).

Prenatal mortality is defined as the total number of infants who died *in utero* after 28 weeks of gestation or during their first week of life, irrespective of gestational age. Autopsy was performed on all deceased infants.

**Statistical calculations.** If not otherwise stated the calculations were made in the following way: fourfold tables were constructed on a pair basis for differences in qualitative

Table III Complications during the pregnancy

	PROM	Controls
Bleeding 1st trimester	9	
Bleeding 3rd trimester	3	1
Infections	5	
Toxaemia of pregnancy	0	1
Polyhydramnios	1	1
Premature contractions	6	6
No complications	10/30	17/16

variables and the hypothesis equal or higher in the control group was tested against the hypothesis higher frequencies in the observed group using the binomial (McNemar's) test (1).

## RESULTS

### Mothers

**Frequency.** During the period 3391 per cent delivered in the Department. Thirty per cent PROM were admitted corresponding to a frequency of 0.9 per cent.

**Previous gynecological history.** Forty per cent (primary or secondary involuntary sterility) preceding the last pregnancy were 23 per cent of the PROM patients as compared with 7 per cent among the controls. The difference was significant statistically, however, as previous genital infections were not represented among the PROM patients.

10

Number of patients

6

29 30 31 32 33 34  
Week of gestation

Fig. 2 Time incidence of premature rupture of membranes.

Table II Complications in previous pregnancies

	PROM	Controls
Bleeding	0	2
Toxaemia of pregnancy	5	2
Premature contractions	2	3
PROM	4	1
No complications	6/19	13/19

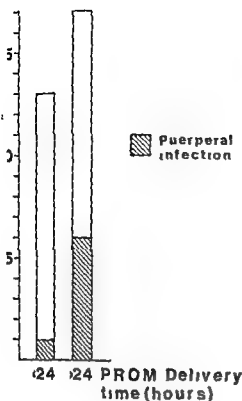


Fig. 4. Puerperal infection in relation to PROM delivery.

ing the controls. Only one of the vaginally treated PROM patients (4 per cent) had manual removal of the placenta. The average frequency of type of intervention in the Department during the study was 1.5 per cent.

**Puerperal infections.** A patient with clinical signs of puerperal infection was considered puerperally infected if her body temperature measured  $\geq 38^\circ\text{C}$ . On two occasions separated by one hour or more. All cases of puerperal and/or neonatal infection are listed in Table IV.

The incidence of puerperal infection in the PROM group was 27 per cent. Patients with PROM delivery time  $> 24$  h developed puerperal infection in 6 per cent of the cases, as compared with 8 per cent in patients with a PROM delivery time  $< 24$  h; the difference being statistically significant ( $p < 0.05$  by Fisher's exact test). Compared with the controls who had no puerperal infections, the PROM patients delivered  $> 24$  h after PROM had a significantly increased incidence of puerperal infection ( $p < 0.001$ ). The relation between PROM delivery time and puerperal infection is illustrated by Fig. 4. As could

be expected, the majority of puerperal infections occurred in patients with a PROM delivery time  $> 24$  h. No prophylactic antibiotics were used, therapy being instituted only when clinical signs of amnionitis appeared. All cases of puerperal infection reacted well to antibiotic therapy.

### Infants

The group of neonates studied consisted of 18 boys, one stillborn, 16 girls, and included four sets of twins. Birth weights varied between 950 and 2730 g. Two babies, each a twin, were small for date, while 2 infants were large for date, the rest being of a size appropriate for their gestational age. Neonatal asphyxia (i.e. an Apgar score of 6 or less at 5 min) was recorded in 5/33 (15 per cent) newborn babies.

IRDS was diagnosed in 3 (9 per cent) infants (Table IV). Two of these died, each a twin. Both infants received treatment with CPAP (continuous positive airway pressure via a nasal cannula) and later with respirator. The twin sister of the latter died of intraventricular and pulmonary hemorrhage. One of 3 infants with IRDS survived. Fourteen (42 per cent) other babies had varying causes (transient tachypnoea 11, pneumothorax 1, Wilson Mikity 1, meconium aspiration 1) and degrees of respiratory distress and received oxygen therapy. The outcome was favorable in these cases.

Septicaemia was diagnosed in 4 babies (Table IV) and was suspected but not confirmed in 2 other infants.

One aforementioned twin died of *Klebsiella* sepsis in connection with IRDS, another of combined *Klebsiella* and group B streptococcal sepsis. In the latter case the streptococcal pathogen was isolated from the mother's cervix as well, and she also had clinical signs of puerperal infection.

Three of the 4 confirmed cases of sepsis were found among infants with no longer than median (25 h) duration of PROM. This distribution did not reach statistical significance, however.

Only one of the 4 mothers of infants who later had sepsis had an elevated body temperature ( $38^\circ\text{C}$  or more) perinatally. Another of these mothers later developed postoperative infection which necessitated a repeat laparotomy and abscess drainage. In this case, however, the same microorganism could not be traced in both mother and child. Maternal fever preceding delivery was encountered in 6 cases (Fig. 5).

One infant with a birth weight of 950 g and a gestational age of 29 weeks died of asphyxia during a breech delivery. In this case group B streptococci

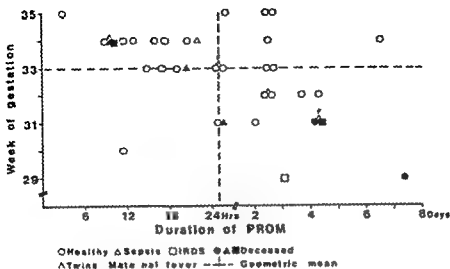


Fig 3 Is 1-10000

were isolated from the mother's cervix and the amniotic fluid as well. No culture was taken from the stillborn infant. An infection of the urinary tract was diagnosed in one patient at 2 weeks of age.

The total perinatal mortality was 6/34 (3 boys and 3 girls) or 17.6 per cent. No other fatal disease occurred during the remainder of the neonatal period (28 days after birth). Fig 5 shows the occurrence of IRDS, sepsis, mortality and maternal fever in relation to the gestational age and duration of PROM.

Infants delivered from the mothers in the control group had no neonatal infections and were all singletons.

**Congenital malformations** One asymptomatic ventricular septum defect, one congenital scoliosis with a hemivertebra and one chondrodystrophia were diagnosed among the PROM cases.

**Twin pregnancies** In the PROM group there were four twin pregnancies (11.8 per cent). The controls were all singletons. The four twin mothers all showed clinical signs of infection. 3 of them 0.5–2.5 hours after PROM necessitating completion of the delivery. The fourth mother having peritonitis after Caesarean section. Three of the 8 twin infants died (37.5 per cent).

## DISCUSSION

The varying incidence of PROM reported from 2 per cent to 32.6 per cent (2) reflects the fact that there is no generally accepted definition of the condition. Commonly it is defined as rupture of the membranes prior to the onset of uterine contractions which thus also includes the full term cases. Since full term cases usually do not constitute any obstetric or pediatric

problems the present study was restricted to PROM cases occurring prior to the 36th week of gestation. Thus the PROM incidence in this material (11.8 per cent) should be viewed in this context.

Several previous reports have dealt with the effects of legal termination on subsequent pregnancies (19, 21, 28, 32). Koller & Eikham (19) found a significantly increased incidence of PROM in a subsequent pregnancy in patients ( $p < 0.001$ ) who had their first pregnancy legally terminated. This association could also be traced in our material but was not statistically supported.

The tendency of patients terminated legally to develop PROM and to deliver prematurely in subsequent pregnancies has been assumed to be a result of cervical incompetence caused by lacerations at the time of operation (19, 28). Johnstone *et al* (19) measured the diameter of the internal cervical os in patients previously operated by suction termination and found significantly greater cervical diameters compared with controls. In support of the above assumption we found a significantly higher incidence of PROM in patients with more than one cervical operation. The PROM group in comparison with the control group ( $p = 0.05$ ) and the same was true of the number of cervical traumas following conization and cerclage rupture ( $p = 0.02$ ). Johnstone *et al* (19) found a smaller cervical diameter in patients where the diameter did not exceed 10 mm whereas larger diameters were encountered when the diameter had been more than 12 mm. These findings support the possibility of more serious sequelae in patients as a result of late terminations. In the present material however no significant differences between early and late legal abortions was found.

ility problems were found to be more frequent in the PROM group. This should probably be considered a late complication of legal termination rather than a primary condition.

Previously reported overrepresentation of twin pregnancies among PROM patients (22) could also be seen in our study. *Polyhydramnios*, however, was noted in one patient only. Naeye *et al.* (25) reported from a large prospective study that 11 per cent of the twin deaths were caused by PROM, while the perinatal mortality rate for twins was found to be 1.5 times that for singletons. In our material, no such corresponding relationships were observed, although a threefold increase in the mortality rate for twins was found.

Furthermore, all the twin mothers became infected during the puerperium. Considering the results found in our material and those of Naeye *et al.* (25), it should be emphasized that the combination of infection in pregnancy and PROM is prognostically a very serious condition.

The association between PROM and a possible reduction in the tensile strength of the membranes has been investigated by several authors with varying results (20-26). Thus, a higher stress tolerance was found in preterm membranes as compared with term specimens. Furthermore, Lavery & Rouse (20) have shown that the stress tolerance of the membranes decreases with advancing gestational age, forming suitable conditions for rupture at term. It has also been demonstrated that the membrane thickness is the application of stress was very similar in term and preterm cases and that in fact preterm membranes tolerated greater stress application than term membranes. Consequently, they concluded that in the case of PROM, despite a basically better stress tolerance, the rupture of the membranes might be caused by some local defect.

It seems reasonable to assume that an anatomical defect of the membranes, such as a marginal cord insertion, might constitute such a local defect. Thus, Naeye *et al.* (8) in 32 cases of marginal cord insertion found a 65 per cent incidence of premature rupture and a 47 per cent incidence of PROM. These results suggested that marginal insertion of the cord causes interference with adequate fetal circulation, thereby restricting nutrition of the fetus, and finally leading to premature labor. It is also conceivable that a partial or velamentous cord insertion, engaging the cervix or less of the adjacent fetal membranes, could make them more friable by reducing their normal blood supply. In our 30 PROM pregnancies we

found 2 marginal and 1 membranous insertion corresponding to a 10 per cent incidence, as compared with 6 per cent in the Brody material (8).

Secondarily acquired defects of the fetal membranes as a result of poor nutritional status have been suggested by Wideman *et al.* (31). They found a relationship between low plasma ascorbic acid levels and PROM in patients coming from lower socioeconomic classes. Probably the low ascorbic acid levels in these patients reflect their generally poor nutritional status.

Meyer *et al.* (23) found another interesting relationship between smoking and PROM, reporting a threefold increase in the risk of PROM for smokers among deliveries before the 34th week of gestation. This finding was verified in our study, where we found significantly ( $p=0.01$ ) more heavy smokers in the PROM group than among the controls.

Almost 30 years ago Knox & Hoerner (17) were able to demonstrate degenerative changes at the site of rupture in membranes from patients delivered prematurely. They assumed that these local defects of the membranes were caused by ascending infection, which made them inflamed and friable. They also suggested that the infection could result in an increased irritability of the uterus, thus initiating contractions and finally PROM. Their paper, however, did not include microbiological studies. Since then many investigators have tried to elucidate the microbiological flora in patients with PROM in order to reveal organisms responsible for this clinical condition and for the possibly occurring puerperal and neonatal infections. These attempts, however, have not adduced any indisputable experimental support for the theoretically attractive hypothesis of Knox & Hoerner. On the other hand, the significance of ascending infection for the maternal and especially the neonatal outcome has been well documented (2, 4, 7, 12, 13, 24, 27).

In clinical obstetrics an ominous sign of impending amnionitis following rupture of the membranes is the occurrence of maternal fever. According to Bobitt *et al.* (7), maternal fever could, however, often be a late sign of ascending infection. In their material they frequently encountered microbiological evidence of amnionitis without any maternal fever. In our study we found maternal fever to be an unreliable prognostic indicator. The clinical course for the 6 mothers with fever was uncomplicated, and only one of the 4 cases of neonatal sepsis came from this group.

In the microbiological part of this study (12) we

have suggested that the significance of anaerobic bacteria in puerperal infections is probably greater than has earlier been realized. Thus the total number of anaerobic isolates from the cervix outnumbered the aerobic ones both in the PROM group and among the controls. It should be emphasized that we were able to isolate the generally pathogenic *Bacteroides fragilis* from the cervix, amniotic fluid or placenta in 23 per cent of the PROM patients but not in a single case among the controls. No correlation between these findings and the occurrence of puerperal infection could be established in this material however. The findings of Del Bene *et al* (11) of a decrease and increase in the number of aerobic and anaerobic strains respectively on comparing patients with PROM after < 12 versus > 12 hours also suggest the importance of the role of anaerobes for the subsequent clinical course. Further investigations to elucidate the significance of the anaerobes as causative or complicating agents are in progress.

The significance of the interval between PROM and delivery has been a matter of endless discussion. As is the case in this as well as in previous studies (2, 13, 24) the risk of puerperal infection increases with the length of the latent period. Although in our material the majority of puerperal infections followed a favorable course there are reports of severe complications and even maternal deaths (4) which call for attempts to shorten the latent period. On the other hand it is also necessary to take into consideration the neonatal risks caused by prematurity. An active policy favors the outcome in terms of maternal and neonatal infection (2). On the other hand there are reports suggesting a lower incidence of IRDS after prolonged rupture of the membranes (3, 6, 24) which could possibly speak in favor of a conservative routine in selected cases. The low incidence of IRDS in our present material does not allow of any conclusions as to the association of PROM with a reduced frequency of IRDS. Earlier as well as recently published studies present contradictory results leaving the issue open to debate (2, 3, 6, 15, 24).

In the present material there was a high perinatal mortality (17.6 per cent). Mortality rates between 4.1 and 20 per cent are found in comparable studies (2, 6, 10, 13). The present relatively high mortality rate should be considered in the light of the fact that all the neonates lost had low birth weights, between 950 and 1 950 g (mean 1 335 g). A correspondingly high mortality rate for infants in the 1 000–2 500 gram

range (15.1 per cent) has been reported by Goss *et al* (13). The overall morbidity in this study was relatively high, only 9/33 infants (27 per cent) had an uneventful neonatal period, not requiring intensive incubator care and phototherapy. Pyles *et al* (10) reported an 8 per cent and Knudsen *et al* (11) a 10 per cent incidence of proven sepsis among premature babies born after PROM, as compared to 10 per cent in the present material. No positive correlation was found between neonatal sepsis and prolonged rupture of the membranes in the present study.

It should be mentioned that in 3 out of 4 cases of neonatal sepsis the etiological agent had already been isolated from nasal swabs taken from the mother immediately after delivery (12) which allowed the institution of early therapy.

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Submitted for publication January 21 1980

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## AUDITORY EVOKED RESPONSES OF THE HUMAN FETUS

## I Behavior during progress of labor

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ly studies demonstrate that the human fetus can and react to auditory stimulation during pregnancy (1 5 6 8 10 12 14 15 20). Modifications of reaction during labor have been described by etta (20) Barden (1) Hon (10) and Rosen (16 17 19) have shown that this behavior involves reaction of stimuli resulting in an increase in the neural activity of the fetal brain and that modifications of these patterns are elicited by several stimuli by labor trauma Hon (10) Garcia Aust (7) and n (18) have attempted to correlate electroencephalographic with fetal heart rate changes. These studies suggest that auditory stimulation and evaluation of the fetal responses offer a new approach to the investigation of at least one function of the brain during pregnancy and labor. The approach has the advantages of being harmless providing an immediate result and needing unsophisticated instrumentation. There is a lack of consistency in published reports probably caused by differences in the type and intensity of the stimulus presented to the fetus. This was confirmed by our early experience (13). It seemed logical to us that a suitable stimulus applied with a consistent technique should elicit comparable responses. Using this method we were able to discriminate between the responses of fetuses in a favorable environment those in an unfavorable environment and those stimulated by labor. Similar results have been obtained by Read and et al (15) who used a related procedure for investigating fetal condition in high risk pregnancies. They based their conclusions on the absence or presence of fetal heart rate acceleration. We used several other parameters of the fetal heart rate response and find that these provide fuller information on fetal condition following a sound stimulus. We have termed this procedure the *Auditory evoked response*.

This paper deals with our observations of the auditory evoked response during labor. The data came from a series of 180 unselected pregnant women whose labor was monitored electronically. This paper is divided into two parts. The first describes the modifications observed in the auditorily evoked response with progress of labor. The second part deals with several factors which influence the pattern of the fetal response. In both studies the fetuses were divided into those who were vigorous at birth (Apgar score 7-10) and those who were born in a depressed condition (Apgar score 1-6). These groups will be called *vigorous* and *depressed* fetuses respectively. We plan to investigate the children psychologically to ascertain any possible significance of the variations observed in the auditory evoked response and to try to correlate this new test of cerebral function in the fetus with postnatal mental development and behavior.

## MATERIAL

Sound stimulation was delivered to the fetus through the maternal abdomen by a modified two-inch flat tweeter. The frequency used was 1 500 cycles per second and the output was 125 decibels (measured at the driver's surface). The stimulus was generated by a specially built oscillator amplifier which provided 5 pulses lasting 3 seconds each separated by one second intervals. During the procedure the

Table I *Motor responses (all stimuli)*

	Response		
	Absent	Mild	Intense
Newborn			
Depressed	19	27	17
Vigorous	42	138	133
	61	165	150
			313
			376

 $\chi^2 = 12.24$  with two degrees of freedom  $p < 0.01$



Table II Motor responses and progress of labor in the group of vigorous newborns

Motor response	Cervical dilatation in cm		
	1-3	4-7	8-10
Absent	7	11	34
Mild	35	56	47
Intense	31	64	39
	73	131	109

$\chi^2 = 12.11$  with 4 degrees of freedom  $p < 0.07$

Table III Motor responses and progress of labor in the group of depressed newborns

Motor response	Cervical dilatation in cm		
	1-3	4-7	8-10
Absent	3	8	1
Mild	10	8	9
Intense	7	7	3
	20	23	13

$\chi^2 = 4.58$  with 4 degrees of freedom (not sig. diff.)

fetal heart rate and uterine contractions were monitored by a suitable fetal monitor (H P 8030 A Corometrics 101 or 111 B) modified to record the pulses of sound stimuli. Only patients in active labor were used for this study which was undertaken between November 2nd 1974 and December 27th 1977. One hundred and eighty cases from the private practice of two of the authors were studied: the only criteria for selection being the availability of the apparatus and of the personnel involved in this research. This group contained many high risk patients. We tried to apply not less than three sets of stimuli to each case: at 1-3 cm at 4-7 and after 8 cm of cervical dilatation. This was not always possible because of clinical conditions. A total of 376 sound stimuli were applied with a mean of 2.09 sets of stimuli per case.

In the interpretation of fetal responses to sound stimuli, we have evaluated the

- 1) motor responses
- 2) heart rate responses

## METHODS

1) Motor responses: Sound stimulation in depressed newborns always produced an active motor response from the 1st trimester of normal pregnancy (NPL 13) and in 81.1 per cent of fetuses studied during labor (Table I). This motor response consists primarily of strong and sudden fetal activity similar to a Moro reflex in the newborn. This is followed by a definite increase in fetal activity which frequently lasts for 30 minutes or more. This reaction is associated with a fetal heart rate response but lasts longer. The motor movements coincide with transient fetal heart rate acceleration.

Table IV Comparison of measured values between the groups of vigorous/depressed newborns (all cases)

Measured value	Vigorous newborns	Difference	Depressed newborns
<i>Duration of fetal heart rate response</i>			
Sec	223.71		116.68
S.E.	11.93		11.34
Number	308		61
Difference t value	5.51		
Difference significance	p < 0.000001		
<i>Difference between mean values of FHR before and after stimulation</i>			
Beats/min	9.86		5.94
S.E.	0.72		0.67
Number	144		63
Difference t value	3.81		
Difference significance	p < 0.001		
<i>Initial increase in fetal heart rate</i>			
Beats/min	20.98		14.94
S.E.	0.74		1.17
Number	309		64
Difference t value	4.33		
Difference significance	p < 0.0001		
<i>Maximal increase in fetal heart rate</i>			
Beats/min	28.91		11.52
S.E.	0.92		1.79
Number	283		64
Difference t value	5.33		
Difference significance	p < 0.000001		

Table V Measured values of FCF and progress of labor in vigorous newborns

Measured values	Cervical dilatation 1-3 cm	Diff	Cervical dilatation 4-7 cm	Diff	Cervical dilatation 8-10 cm
<i>Duration of fetal heart rate response</i>					
sec mean	295.93		230.71		167.45
S.D.	231.65		181.92		211.62
S.E.	27.30		16.21		20.36
Number	72		126		108
Difference significance	$p < 0.05$ $p < 0.02$ $p < 0.001$				
<i>Difference between mean values of FHR before and after stimulation</i>					
beats/min mean	12.67		8.36		6.68
S.D.	7.15		8.64		9.04
S.E.	1.26		1.01		1.35
Number	32		73		45
Difference significance	$p < 0.01$ $p < 0.002$ $n.s.$				
<i>Initial increase in fetal heart rate</i>					
beats/min mean	24.07		22.54		17.74
S.D.	12.64		12.31		13.29
S.E.	1.50		1.12		1.27
Number	70		117		97
Difference significance	$n.s.$ $p < 0.01$ $p < 0.01$				
<i>Maximal increase in fetal heart rate</i>					
beats/min mean	32.33		28.63		25.82
S.D.	13.32		15.81		16.44
S.E.	1.59		1.46		1.67
Number	70		117		97
Difference significance	$n.s.$ $p < 0.01$ $n.s.$				

which continue even when the basal fetal heart rate returned to pre stimulation values. The intensity of the response varies from case to case so that a simple classification of present/absent seemed insufficient. The classification of the intensity of the motor response was described in the previous publication and has been used in this study. It is as follows:

*Motor response* a strong sudden generalized movement of the fetus easily recognized by the observer or felt by the mother usually followed by a prolonged period of fetal activity.

*Weak motor response* a movement felt by the mother or observed by the attendant which could be sudden but was strong usually not followed by any detectable increase in fetal activity.

*No motor response* no movement detected by the observer or felt by the mother.

*Fetal heart rate responses* The fetal heart rate changes were noted by direct reading of the records from the fetal monitor. The following items were measured: *duration of fetal heart rate acceleration*, *differences between means initial rate of fetal heart rate and maximal increase of fetal heart rate*.

*Duration of fetal heart rate acceleration* We always obtain a fetal heart rate acceleration response (Fig. 1) when the fetus was not depressed. The duration of the response was measured on the record in min from the beginning of the stimula-

tion to the point where the fetal heart rate returned to the pre-stimulation level for a steady period of not less than 5 min (30 seconds). The measured values have been converted into time in seconds to facilitate the calculations.

*Differences between means* The basal fetal heart rate was determined for at least five minutes before stimulation by taking the mean of the values at each peak on the fetal heart rate tracing. The same procedure was continued for the period of acceleration following stimulation and the values obtained were compared statistically using Student's *t* test for dependent samples.

*Initial increase of fetal heart rate* This was the difference between the fetal heart rate immediately preceding stimulation and the first peak on the record after stimulation. The strong movements of a vigorous fetus sometimes hamper the analysis of this parameter when the fetal heart rate is monitored by external ultrasound techniques.

*Maximal increase of fetal heart rate* This was the difference between the greatest value attained by the fetal heart rate during the period of acceleration and the value immediately before stimulation.

Besides these responses we have occasionally observed a sudden deceleration of fetal heart rate at the beginning of the response; this was sometimes associated with entanglement of the cord around the fetal neck. This finding was too infrequent to warrant detailed analysis. The same applies to the increased frequency of transient accelerations observed after sound stimulation. Such accelerations are additional

Table VI Measured values of FCF and progress of labor in depressed newborns

Measured values	Cervical dilatation 1-3 cm	Diff	Cervical dilatation 4-7 cm	Diff	Cross data
<i>Duration of fetal heart rate response</i>					
Sec	167.21		129.78		ns
S.D.	160.62		112.56		ns
S.E.	36.83		23.47		ns
Number	19		23		ns
Difference significance	ns		p < 0.01		
<i>Diff. between mean values of FHR before and after stim</i>					
Beats/min	7.19		6.66		ns
S.D.	8.90		6.39		ns
S.E.	2.16		1.36		ns
Number	17		23		ns
Difference significance	ns		p < 0.01		
<i>Initial increase in fetal heart rate</i>					
Beats/min	17.55		15.91		ns
S.D.	12.01		10.76		ns
S.E.	2.68		2.24		ns
Number	20		23		ns
Difference significance	ns		p < 0.01		
<i>Maximal increase in fetal heart rate</i>					
Beats/min	23.53		21.04		ns
S.D.	15.79		14.10		ns
S.E.	3.62		2.94		ns
Number	19		23		ns
Difference significance	ns		p < 0.01		

evidence of fetal wellbeing but during labor they can be caused by many other factors so that numerical analysis is not possible.

## RESULTS

**Motor responses.** We have analyzed 376 stimuli of which 313 were applied to the group of 151 vigorous fetuses and 63 to the group of 29 depressed fetuses. Table I shows that the proportion of absent responses was greater in the group of depressed fetuses than in the group of vigorous fetuses. A Chi square test indicated that the differences were significant at a level of  $p < 0.01$ .

We investigated whether the progress of labor could influence the pattern of the motor response. For this we compared the data for each group (vigorous or depressed) with the degree of cervical dilatation (Tables II and III). The number of absent responses increased with the progress of labor in the group of vigorous fetuses. A Chi square test was significant at  $p < 0.02$ . No such trend was found in the group of depressed fetuses (Chi square not significant).

**Heart rate responses.** With the kind of stimulus a fetal heart rate response can always be expected was observed in normal or mildly depressed fetuses (Fig 1). This agrees with our previous observations in labor (NPL 13). The response obtained was clearly delineated in all cases where the fetus was not agitated or actively moving. It consisted of acceleration beginning immediately after stimulation, bringing the basal heart rate to a level above the previous rate. This is maintained for usually less than 200 seconds, after which the rate falls back to the previous level. Sometimes the response is marked by an abrupt fall or withdrawal from the steady elevated rate to a closely associated transient acceleration. The heart rate response is considered terminated when the period between each acceleration is longer than more of a steady rate equal to or only slightly above the previous level. It was possible to measure the difference between the mean values of the heart rate before and after stimulation in 34 of the patients.

Table VII Differences in measured values between the groups vigorous/depressed newborns (numbers within parentheses) during progress of labor

	Duration of FHR response sec		Diff between means beats/min		Init increase in FHR response beats/min		Max increase in FHR response beats/min	
	Vig n b	Depr n b	Vig n b	Depr n b	Vig n b	Depr n b	Vig n b	Depr n b
<i>Cervical dilatation 1-3 cm</i>	295 (172)	167.21 (119)	12.67 (37)	7.19 (37)	24.07 (71)	17.55 (20)	32.33 (70)	23.53 (19)
t value		2.81		2.19		2.12		2.22
significance		$p < 0.01$		$p < 0.04$		$p < 0.04$		$p < 0.03$
<i>Cervical dilatation 4-7 cm</i>	30.71 (126)	129.78 (23)	8.36 (73)	6.86 (22)	22.54 (121)	15.91 (23)	28.63 (117)	21.04 (23)
t value		3.54		0.88		2.64		2.31
significance		$p < 0.001$		n.s.		$p < 0.01$		$p < 0.03$
<i>Cervical dilatation 8-10 cm</i>	167.45 (108)	58.91 (22)	6.68 (45)	2.22 (21)	17.74 (109)	10.14 (21)	25.82 (97)	12.29 (21)
t value		4.25		2.94		3.43		4.70
significance		$p < 0.0001$		$p < 0.01$		$p < 0.001$		$p < 0.00001$

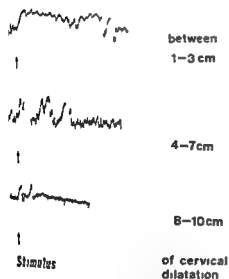
### A COMPARISON BETWEEN GROUPS OF VIGOROUS AND DEPRESSED FETUSES

**Duration of the response** The mean duration of the fetal heart rate response in the group of vigorous fetuses was 223.73 seconds. In the group of depressed fetuses the mean was 116.68 seconds (Student's test gave  $p < 0.0000001$ ) (Table IV). Even considering the great variability in the data, this parameter is very useful when predicting the condition of the neonate at birth (Fig 2).

**Differences between means** The results differed between vigorous and depressed fetuses. In the former group the mean increase of fetal heart rate after sound stimulation was 9.86 beats per minute, while in the group of depressed fetuses it was only 5.58 beats per minute. Student's test gave  $p < 0.001$  (Fig 3).

**Initial increase of fetal heart rate** The initial increase of fetal heart rate in the group of vigorous fetuses amounted to a mean of 20.98 beats per minute, while in the group of depressed fetuses it was 14.39 beats per minute. Student's test gave  $p < 0.0001$  (Fig 4).

**Maximal increase of fetal heart rate** The greatest increase in fetal heart rate after sound stimulation



Example of fetal heart rate response at different stages of labor. Heart rate tracings obtained through scalp electrodes.

Table VIII Comparison of measured values at the end of labor in vigorous newborns and at beginning of labor in depressed newborns

	Vigorous newborns at 8-10 cm of cervical dilatation	Depressed newborns at 1-3 cm of cervical dilatation
Duration of FHR response sec	167.45	167.26
Diff between mean values of FHR before and after stim beats/min	6.68	7.19
Init increase in FHR beats/min	17.74	17.55
Max increase in FHR beats/min	25.82	23.53

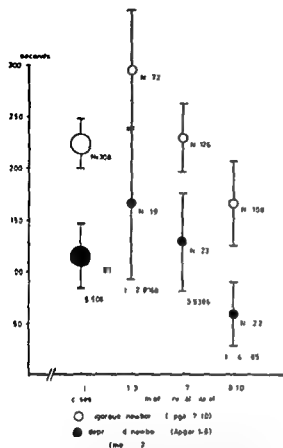


Fig 2 Length of fetal heart rate responses in vigorous and depressed newborns (larger circles all cases smaller circles are values at different periods of labor)

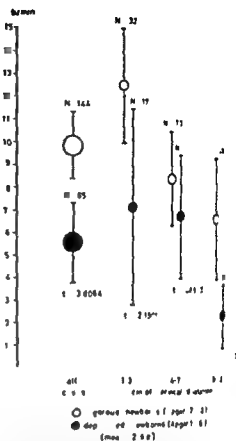


Fig 3 Differences between means in vigorous and depressed fetuses (larger circles all cases smaller circles are values at different periods of labor)

amounted to a mean of 28.91 beats per minute in the group of vigorous fetuses and 18.32 beats per minute in the group of depressed fetuses. Student's test gave  $p < 0.000001$  (Fig 5).

**Comments:** The groups of vigorous and depressed fetuses differed significantly in all the parameters measured. The most discriminating function was the duration of the fetal heart rate response. These observations suggest that fetal reaction to sound stimulation can be used during labor to identify the depressed fetus. The decrease in fetal heart rate response usually precedes other signs of fetal depression and is sometimes the only available predictor of neonatal depression.

## II DIFFERENCES DURING PROGRESS OF LABOR

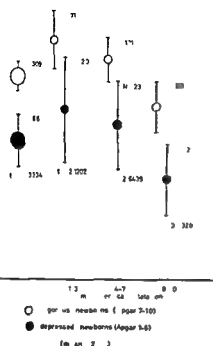
The results were also analyzed within the groups of vigorous and depressed fetuses. These data are shown in Figs 2-5 and in Tables V-VIII.

### Vigorous fetuses (Table V)

**Duration of fetal heart rate response:** The fetal heart rate response decreased significantly as labor progressed. The values were 79.91 seconds at 1-3 cms, 230.71 seconds at 4-7 cms and 160.71 seconds at 8-10 cms of cervical dilatation. Differences between these values were all statistically significant. It is interesting that the mean at 1-3 cms of cervical dilatation in this group of vigorous fetuses was equal to the mean observed at the beginning of labor in the group of depressed fetuses (Fig 1).

**Differences between means:** The differences between means before or after sound stimulation decreased as labor progressed. The values were 10.56 seconds at 1-3 cms, 12.56 seconds at 4-7 cms and 7.00 seconds at 8-10 cms of cervical dilatation. Significant differences were found between the means at 1-3 and 4-7 cms ( $p < 0.01$ ) and between those at 1-3 and 8-10 cms ( $t = 3.2411$ ,  $p < 0.002$ ) (Fig 3).

**Initial increase of fetal heart rate response:** The initial increase of fetal heart rate response decreased with progress of labor. The values were 1.56 beats per minute at 1-3 cms, 1.56 beats per minute at 4-7 cms and 1.56 beats per minute at 8-10 cms of cervical dilatation. Significant differences were found between the means at 1-3 and 4-7 cms ( $p < 0.01$ ) and between those at 1-3 and 8-10 cms ( $t = 3.2411$ ,  $p < 0.002$ ) (Fig 3).



Initial increase of fetal heart rate in vigorous and depressed fetuses (larger circles all cases smaller circles are values at different periods of labor)

stages were 24.07, 22.54 and 17.74 beats per minute. Statistically significant differences were found between the means at 1-3 and 8-10 cm ( $t=2.178$ ,  $p<0.01$ ) and between those at 4-7 and 8-10 cm ( $t=2.8315$ ,  $p<0.01$ ) (Fig 4).

**Initial increase of fetal heart rate.** This measure of fetal heart rate response fell steadily as labor progressed but not as markedly as the other parameters measured. A significant difference was found between the values at 1-3 and 8-10 cm of cervical dilatation ( $t=2.8227$ ,  $p<0.01$ ) (Fig 5).

#### Depressed fetuses

**Duration of fetal heart rate response.** The fetuses that were born depressed had a shorter duration of fetal heart rate response in early labor compared with those that were vigorous and also showed a more marked decrease in the response as labor progressed. At the beginning of labor the response lasted 167.21 seconds, fell to 129.78 at 4-7 cm of cervical dilatation and to 91.91 seconds at 8-10 cm. The latter amounts to

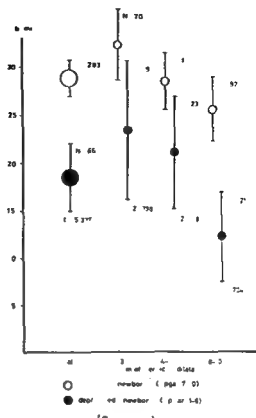


Fig 5 Maximal increase of fetal heart rate in vigorous and depressed fetuses (larger circles all cases smaller circles are values at different periods of labor)

only 35.23 per cent of the initial response while in the group of vigorous fetuses the response at the end of labor was 56.58 per cent of the initial response. Significant differences were observed between 1-3 and 8-10 cm ( $t=2.7110$ ,  $p<0.01$ ) and between 4-7 and 8-10 cm ( $t=2.1915$ ,  $p<0.04$ ) (Fig 2).

**Differences between means.** Similar trends were observed for this measurement of the fetal heart rate response, the values being 7.19, 8.86 and 2.22 beats per minute. Despite a great range and the relatively small number of cases studied, significant differences were found between the means at 1-3 and 8-10 cm ( $t=2.1915$ ,  $p<0.04$ ) and between those at 4-7 and 8-10 cm ( $t=3.0352$ ,  $p<0.01$ ) (Fig 3).

**Initial increase of fetal heart rate.** The same progressively decreasing pattern of response intensity was observed for this function. The mean values were 17.55, 15.91 and 10.14 beats per minute at the three periods of labor studied. A significant difference was observed only between 1-3 and 8-10 cm of cervical dilatation ( $t=2.2801$ ,  $p<0.03$ ) (Fig 4).

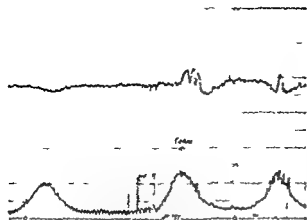


Fig 6 Example of fetal heart rate acceleration elicited by touching cephalic presentation in absence of heart rate response to sound stimulation

**Maximal increase of fetal heart rate** The mean values for this function also diminished with progress of labor. They were 23.53, 21.04 and 12.29 beats per minute for the periods of labor considered. Significant differences were observed between 1–3 and 8–10 cm ( $t=2.6054$ ,  $p<0.02$ ) and between 4–7 and 8–10 cm of cervical dilatation ( $t=2.3279$ ,  $p<0.03$ ) (Fig 5).

### C DIFFERENCES BETWEEN THE GROUPS OF VIGOROUS/DEPRESSED FETUSES DURING PROGRESS OF LABOR

There were statistically significant differences between the groups of vigorous and depressed fetuses for all the parameters studied. A comparison was made between the mean values of each measurement for the groups of vigorous and depressed fetuses at each period of labor.

**Duration of the fetal heart rate response** The differences between the groups were 128.72 beats at 1–3 cm, 100.93 beats at 4–7 cm and 108.54 beats at 8–10 cm of cervical dilatation. All these differences were statistically significant ( $t=2.8068$ ,  $p<0.01$  at 1–3 cm,  $t=3.5386$ ,  $p<0.001$  at 4–7 cm and  $t=4.2485$ ,  $p<0.0001$  at 8–10 cm). Although the numerical differences do not become larger with the progress of labor, their significance increases.

**Differences between means** The inter group differences were 5.11, 1.5 and 4.46 beats at the three stages of dilatation. Although this is not the best parameter for differentiating the vigorous from the depressed fetuses, significant differences were observed at 1–3 cm and at 8–10 cm ( $t=2.1901$ ,  $p<0.04$  and  $t=2.9425$ ,  $p<0.01$  respectively).

**Initial increase of the fetal heart rate** The differences between vigorous and depressed fetuses were 6.63 and 7.6 beats per minute at 1–3 cm ( $t=2.1202$ ,  $p<0.04$ ), 2.6433,  $p<0.01$  and 1.18,  $p<0.001$ ) at the three stages of labor.

**Maximal increase of fetal heart rate** Significant differences were observed between vigorous and depressed fetuses. At 1–3 cm the difference was 1.11 ( $t=2.2238$ ,  $p<0.03$ ), at 4–7 cm 7.53 ( $t=2.3118$ ,  $p<0.03$ ) and at 8–10 cm 13.11 ( $t=4.7047$ ,  $p<0.0001$ ).

### DISCUSSION

The assessment of damage suffered by the fetus during labor or from depression during pregnancy is one of the problems of modern obstetrics. The most important organ at risk, the fetal brain, is difficult to investigate. Electroencephalographic recordings of human fetuses have been obtained, but related with fetal brain damage (7). This technique is complex and impracticable even for research.

It is known that a sound stimulus elicits electrical activity in the fetal brain (1). In studies in the fetus, in the newborn and in the adult, it has been shown that this is followed by a heart rate acceleration (2). Brazelton (4) has shown the behavioral response to external stimuli provide a most sensitive method to evaluate the central and peripheral nervous system of human infants. Probably this is also true for the fetus. The study of this fetal adaptation is

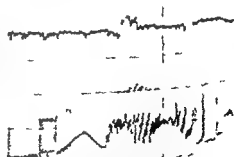


Fig 7 Fetal heart rate acceleration elicited by increase of intra abdominal pressure due to crowding when no response was obtained to external stimuli (Both examples from same case of a 32-week born Argar 4/10 in hours delivery).

is a useful approach in the study of fetal condition before birth

the analysis of data presented in this paper the facts need discussing. The first is the decrease in auditory evoked response with the progress of labor. This occurs even when the fetus is going to be born vigorous and apparently normal and tests that some kind of change in cerebral function occurs even under physiological conditions. This decrease of the auditory evoked response is also present in many conditions where fetal depression is known to occur such as in cases of cephalo-pelvic disproportion, premature rupture of the membranes, small-for-dates syndrome and in preeclampsia and others have previously described the absence of response when the fetus is seriously depressed.

(5) A careful follow up of the present infants will help to clarify the meaning of these observations. Secondly, it seems that the fetal auditory evoked response involves a sensitive parameter of fetal brain activity. In cases of mild fetal depression where no response can be obtained, a response in the form of fetal heart rate acceleration and fetal movement still be elicited by other external stimuli such as vaginal touching, touching the fetal presentation. This is demonstrated in Figs 6 and 7.

Another point that can be considered is the differences observed in the development of the motor auditory evoked response in vigorous compared with depressed fetuses. With vigorous fetuses the response decreases with the progress of labor while with depressed fetuses the response is already decreased at the beginning of labor and does not change with progress of labor. This suggests that the mechanisms producing fetal heart rate and the motor responses are relatively independent as they behave dissimilarly in group.

Finally, it must be stressed here is that the depressed fetal auditory evoked response can give other signs of fetal distress. An impaired response can sometimes be identified during pregnancy at the beginning of labor. The response of the fetus to acoustic stimulation may provide a new and relatively simple way of assessing the condition of the fetus. The following points are important:

1) fetal monitors are available in the majority of well-equipped hospitals

2) sound stimulators are easy and cheap to build or adapt

3) the perception and response to sound stimulation functions of cerebral cortex as already dem-

onstrated by fetal or neonatal electroencephalograms

4) the human fetus has been demonstrated to hear from 24 weeks of pregnancy, a period of gestation when a poor intrauterine environment may begin to affect its development (12)

5) the fetal heart rate and motor responses are parameters of fetal condition

6) the method can be used during pregnancy since the procedure is non-invasive

7) the test can be repeated several times since it causes no harm to the mother and is much less time-consuming than the oxytocin challenge (Pose test) or similar procedures

8) at the frequency used, the attenuation of noise by the maternal abdomen ensures that no harm will result to auditory organs of the fetus since the noise reaching the amniotic fluid is several decibels below the normal sound levels in the intrauterine environment (21)

We have termed this test 'auditory evoked response' in spite of the fact that the term 'evoked response' is more often associated with electroencephalography. This nomenclature was used in our introductory paper on the subject (14). The behavior of the auditory evoked response during complications of labor will be presented in part two of this paper. A third paper will deal with this new test during pregnancy relating it to the oxytocin challenge (Pose test) and to the outcome of pregnancy.

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*Submitted for publication April 28 1977*

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# CLINICAL AND ULTRASONIC ASPECTS IN THE DIAGNOSIS AND FOLLOW UP OF PATIENTS WITH EARLY PREGNANCY FAILURE

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The value of some central clinical and ultrasonic signs at the time of threatened abortion was examined in patients in the 6th-20th weeks. The outcome of the pregnancy was delivery in 45 per cent of all the cases. A sign of the first bleeding of 3 days or more predicted a significantly more often than did a shorter bleeding episode. A positive detection of fetal life signs by sound signified a successful outcome for the pregnancy per cent of the patients. The anamnestic duration of pregnancy often seemed to deviate from the real weeks requiring correction by ultrasonic methods. Forty-eight per cent of the cases with various pathologic forms of early pregnancy were evacuated on the basis of ultrasonic findings without waiting for the unavoidable spontaneous abortion. The later course of the pregnancies with a successful outcome revealed an 8 per cent frequency of premature deliveries signifying careful observation of the patients in this respect. The relatively high frequency of fetal mortality (3.4 per cent) seemed to be associated with the prematurity.

The symptoms of early pregnancy failure — uterine bleeding and contractions — constitute a very common diagnostic problem during pregnancy. The most important matters to be settled in this connection are firstly the confirmation of pregnancy, secondly its viability and thirdly specification of the prognostic factors for the outcome of the pregnancy. Since 60 per cent of these pregnancies will end in spontaneous abortion (7, 8) and the frequency of chromosomal anomalies in the abortive tissues is 1-60 per cent (12), an early and reliable differentiation between normal and pathologic pregnancies is very important. Only after this differentiation can the controlling and possible therapeutic measures be applied to the cases in which a normal outcome of the pregnancies can be expected.

During the last ten years the ultrasonic methods have attained a central role in the diagnosis of problems in early pregnancy. The detection of the gestational sac in the uterus from the 6th week onwards (4)

the reliable diagnosis of fetal life signs in the 8th-10th week (9, 15, 17) and the differentiation of the pathologic forms of early pregnancy from the normal ones (20) which have been rendered possible by these methods have opened up entirely new possibilities for the clinician in solving the problems considered here. After that the main task is to predict the subsequent prognosis of the pregnancy. The first observations seemed to signify a successful prognosis for 90 per cent of the pregnancies after threatened abortion if the fetal life signs can be detected (9, 10). In the older reports the chief perinatal complications (increased perinatal mortality, prematurity and congenital malformations) seemed to occur significantly more often in pregnancies complicated with threatened abortion than in control pregnancies (2, 11, 21). Later observations, however, have only confirmed the increased frequency of prematurity, establishing the other pathologic parameters as secondary consequences due to the premature delivery (13).

The aim of this study is to present some central clinical and ultrasonic aspects of the actual diagnostics of early pregnancy failure and some follow-up observations until the termination of pregnancy.

## MATERIAL AND METHODS

The series consisted of 525 patients admitted to the Department of Obstetrics and Gynecology, University of Oulu during about 4 years from 1973 onwards. All the patients had uterine bleeding in the 6th-20th week of pregnancy. The cases where the bleeding seemed to originate from cervical polyps or erosion and no hormonal or histopathological verification of pregnancy could be made were excluded. The series included 7 ectopic and 4 molar pregnancies. All the cases were followed up until abortion or delivery. Twelve per cent of the cases were outpatients while the others were followed up in the hospital. The treatment included only bed rest and symptomatic drugs.

After the recording of anamnestic data and the clinical examination, an ultrasonic examination (by one person) was

Table I Ultrasonic findings at the first examination

	Detection of fetal life	Empty gestation sac	Indistinct intrauterine echoes no gestation sac or fetus	Fetal echoes in gestation sac or in uterus, no gestation sac	Maternal blood	Outcome
Number	202	142	92	75	4	1
Percent	38	26	18	14	1	1

performed on all the patients within the first two days after admission. Therefore the cases with immediate spontaneous abortion were mainly excluded. The main purpose of the ultrasonic examination was to detect the fetal life signs by combining the A-Doppler and TM methods with B scanning (Aretz Combison 400). Real time equipment (Vidoson) was also used on some patients. The examination was repeated once in 154 cases and twice in 71 cases. This was done mainly because of anamnestic uncertainty or clinical discrepancy concerning the duration of pregnancy or to confirm an initially negative finding. The duration of pregnancy was checked by measuring the mean diameter of the gestation sac and/or the biparietal diameter of the fetus.

The statistical analyses were made using Student's *t* test.

## RESULTS

The mean duration of pregnancy was  $10.7 \pm 3.2$  weeks at the beginning of the bleeding and  $11.5 \pm 0.8$  weeks at the first ultrasonic examination. The mean duration of bleeding before the first examination was  $4.0 \pm 5.5$  days. For 75 per cent of the patients followed up in the hospital the treatment was continued for 1-7 days.

Thirty-two per cent of the cases had one or more spontaneous abortions in their anamnesis and 7 per cent had had legal induced abortions. For 45 per cent (233 cases) that pregnancy was the first.

As regards anamnestic data the main interest was directed to the time of beginning and the duration of the first bleeding episode. If this occurred within weeks 5-9 the outcome was abortion in 51 per cent, the corresponding figure for the 10th-14th weeks

being 53 per cent and that for the 15th-20th weeks 69 per cent. These differences were not statistically significant. If the duration of the first bleeding episode was 1-2 days the outcome was abortion in 47 per cent compared with 56 per cent if it was 3 days or more. This difference was not statistically significant ( $p < 0.05$ ). A history of either spontaneous or legal abortions had no significant effect on the outcome of the pregnancy compared with other patients.

The ultrasonic findings at the first examination are presented in Table I. Fetal life was detected in 38 per cent. An empty gestation sac was the most typical finding of a blighted ovum was observed in 26 per cent. Indistinct intrauterine echoes or no gestation sac or a fetus representing a missed abortion were detected in 18 per cent of the cases. Fetal echoes without the diagnosis of threatened abortion were seen in 14 per cent.

The final outcome in the whole series was 45 per cent (Table II). When fetal life was not detected at one of the ultrasonic examinations (87 per cent) the pregnancy ended in delivery in 11 per cent (87 per cent).

Table II The outcome of pregnancies after threatened abortion

	Delivery	Spontaneous abortion	Induced abortion	Hydatidiform mole
No.	236	278	7	4
%	45	52	1	1

Weight of baby > 600 g

Table III Correlation between fetal life detected at the first ultrasonic examination and the outcome of the pregnancy

Weeks of pregnancy	Detection of fetal life			No. of cases		
	No.	Delivery	Abortion	No.	Delivery	Abortion
6th	0	0	0	5	0	5
7th	2	0	0	4	1	3
8th	10	100	0	4	1	3
9th	24	90	10	4	1	3
10th	33	87	13	4	1	3
11th	37	93	7	3	1	2
12th	20	90	10	4	1	3
13th	5	80	16	4	1	3
14th	18	7	21	4	1	3
15th-20th	27	8	18	4	1	3

per cent

#### Table IV Correlation between fetal life detection at second ultrasonic examination and later outcome of pregnancy

No. of patients	Detection of fetal life			No signs of fetal life		
	No	Delivery	Abortion	No	Delivery	Abortion
0	0	0	0	0	0	0
1	0	0	0	4	0	100
2	5	100	0	9	56	44
3	9	89	11	14	29	71
4	11	8	13	8	0	100
5	13	83	17	13	0	100
6	14	93	7	4	0	100
7	20	88	12	5	0	100

weekly prediction for the later course of pregnancy based on fetal life signs can be seen in Table III. Until the 12th week the prognosis is favorable in over 90 per cent if fetal life can be detected at the first examination. If the bleeding has not later (first examination in the 13th week or later) the outcome of pregnancy is abortion in approximately 20 per cent despite the positive detection of fetal life. Yet if no correction is made to the number of gestational weeks (on the basis of the gestation sac or the biparietal diameter) the false positive findings of fetal life signs disappeared only after the 12th week. In Table IV the weeks of pregnancy have been corrected according to the ultrasonic findings at two repeated examinations. This correction: no false negative findings could be derived from the 9th week onwards.

The clinical observations of abortions at the time of uterine evacuation are presented in Table V. Of the patients for whom the outcome was abortion (34 per cent) had the uterine evacuation and curettage performed after information from one or in most repeated ultrasonic examinations revealing the kind of abnormal finding. There was no suspicion of an intact normal pregnancy in these cases at

#### Table V Clinical observations of abortions at the time of uterine evacuation

	Number	Per cent
Spontaneous abortion at home or in hospital	109	37
Only residual tissue in uterus after abortum	42	15
Abortion made after pathological anatomical findings	134	48

#### Table VI The weeks of delivery

	<31th	31th-36th	37th-40th	41th-43rd
No.	9	11	150	66
%	4	4	64	28

the time of the evacuation. In the remaining 52 per cent the abortion occurred spontaneously and curettage was made afterwards. The mean interval between the last negative ultrasonic examination and the abortion was 3.2 days in the whole material. The pregnancy of 31 patients subsequently ended in spontaneous abortion despite the diagnosis of fetal life signs at the time of the symptoms of threatened abortion. The abortion in all these cases occurred in the 12th week or later and the presence of the fetus could be verified in all cases.

The weeks of delivery are presented in Table VI. The frequency of premature deliveries (before the 37th week) was 8 per cent. Seven per cent of the newborns weighed under 2500 g. The rate of perinatal mortality was 3.4 per cent (8 cases). In seven of these cases the main etiologic cause was prematurity. Congenital malformations were observed in 9 newborns (4 per cent). Placenta previa at the time of delivery was diagnosed in 3 cases (1 per cent) but no cases of placental ablation were observed. The rate of manual separation of the placenta was 6 per cent. The sex distribution of the newborns was 119 girls/117 boys.

## DISCUSSION

The distribution of the final outcomes of pregnancies between abortion and delivery in this series corresponded well to earlier observations in which the prognosis has been successful in about 80 per cent of the cases after threatened abortion (8/9/20). However this series included according to the first ultrasonic examination 22 cases (18 per cent) of incomplete abortion in which the clinical judgement was confirmed by ultrasound before the curettage. Thus the truly intact early pregnancies complicated by threatened abortion mostly ended favorably.

The duration of bleeding appeared to have a significant effect on the prognosis of the pregnancy. Bleeding for 3 days or more predicted abortion almost significantly more often than a shorter bleeding episode. This is in accordance with the

earlier observations (8). The week of bleeding onset seemed to have no significant effect although the tendency to abortion increased at a late onset of bleeding. This is in slight disagreement with the earlier observations (1-8) but can be explained by the relatively high number of missed abortions at the first ultrasonic examinations (14 per cent). The bleeding in these cases typically begins during the second trimester. Also abortions seem to be more common in the patients with positive detection of fetal life from the 13th week onwards compared with the earlier bleeding groups.

The most prominent practical finding in the ultrasonic examinations is the prognostic significance of fetal life detection for the later course of pregnancy. In this series this diagnosis signified delivery in 87 per cent. This percentage corresponds to the earlier observations from the same clinic (9-10) and some other results (5-20). This high predictive value accords primary importance to ultrasonic examinations in the diagnostic evaluation of problems in early pregnancy. The interpretation of fetal life findings is simple enough and the results are immediately available for use. The occurrence of false negative results ends by week 10-12 which coincides well with the most common onset of bleeding and the admission of patients for diagnostic procedures. If there is no doubt about the duration of pregnancy or after reliable correction of this all ultrasonic results are correctly positive and negative after the 9th week.

According to the findings made in the first ultrasonic examination the group of blighted ovum is the most common pathologic subgroup (26 per cent) consisting of approximately 30 per cent of all the pregnancies ending in abortion. In this series the ultrasonic scanning was repeated in most of the cases where blighted ovum was suspected at the first examination. If no fetal echoes or life signs were detected in the gestation sac at the second examination either the diagnosis of blighted ovum was confirmed. Thus misinterpretations owing to uncertainty of the duration of pregnancy were avoided and for example a normal finding in the 6th week was not mistaken for a typical blighted ovum in the 10th week. This study also emphasizes the importance of a correct determination of gestational weeks. Since discrepancies of more than 2 weeks between the anamnestic and the real duration of pregnancy have been observed in 40 per cent of the cases in pregnancy materials (3-14) the correct week must be determined by some ultrasonic methods already in early

pregnancy. The most exact results have been obtained with the fetal crown-rump length (3-20) and biparietal diameter ( $\pm 6$  days) (6) and fetal head volume ( $\pm 9$  days) (19). The accuracy of the gestational sac diameter is approximately (16).

After a detection of fetal life the pregnancies ended in later abortion (live abortion) in 11 per cent of the cases in which the existence of the fetus was invariably confirmed. Since the time of abortion in these cases was relatively late, nearly all of them occurred in the second trimester. It is possible that the background of these abortions differs from that of blighted ova. Robinson (20) claims that insufficiency is the main cause of late abortions as opposed to chromosomal abnormalities in earlier abortions. A reliable ultrasonic prediction of late abortions at the onset of bleeding is expected.

In this series the abortion was spontaneous in 52 per cent after the ultrasonic examination. In the other 48 per cent however the uterus was evacuated after one or in most cases several pathologic ultrasonic findings. No doubt about the termination of the intact pregnancies arose in any of these 48 per cent (136 patients). The detection of a pathologic pregnancy with bleeding and pressure for the patient could be a relief. This also involves a notable saving of costs.

The pregnancies which ended in threatened abortion seemed to have a less complicated late course except for the high frequency of premature deliveries. This is approximately twice the control rate 4.7 per cent occurring during the same period. As a consequence of this higher frequency the perinatal mortality rate also increased 3.4 per cent since most of the perinatal losses were among the premature deliveries. This is in accordance with the earlier results. The frequency of premature deliveries was as high (13-20 per cent) as in some earlier series (11-22). This may be due to the recognition of pregnancies as risk cases for the premature delivery according to our observations in the study (10). Hence many patients were followed very closely during the late pregnancy and were admitted to hospital for the prevention of premature delivery.

In conclusion one can state that the use of diagnostic ultrasonic methods to gain information on the current state of the pregnancy and to predict with adequate certainty its late course

of threatened abortion. Owing to the increased risk of premature delivery these patients should be regarded as risk cases during the later stages of pregnancy.

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Submitted for publication August 28 1978

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# ALTERATIONS IN THE ELECTROCARDIOGRAM OF THE FETAL LAMB AS A SIGN OF FETAL ASPHYXIA

A comparison between the scalp lead and the precordial lead

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**Abstract** Progressive changes in the ST-T period of the electrocardiogram (FECG) were studied in 18 lambs acutely exteriorized and subjected to graded hypoxia. The aim of the study was to compare the bipolar precordial lead (CR lead) with the unipolar scalp lead and to correlate the alterations in the FECG to blood gas and acid status. The scalp lead gave less information regarding fetal condition and was more difficult to interpret than the CR lead. This might be one factor in the controversy regarding the significance of alterations in the FECG during hypoxia and labor, since the scalp lead is used mainly in clinical situations. Our previous results demonstrating progressive changes in the ST-T period of the FECG during hypoxia in experimental animals and showing the same changes in newborn human infants immediately after birth were registered with the bipolar precordial lead. It is probable that a bipolar scalp lead might give more information regarding the fetal condition than the unipolar scalp lead mainly used in clinical practice.

Alterations in the fetal electrocardiogram (FECG) as a sign of intrauterine asphyxia and its diagnostic value are controversial. Although alterations in the ST-T period of the FECG have been detected during experimental conditions in human previsible fetuses (2) and during labor in term fetuses (1-11) these alterations are believed to be preceded by changes of the fetal heart rate (FHR) (1-3, 9-10). Our own animal experiments have shown progressive changes in the ST-T period during graded hypoxia and these hypoxic changes occurred well in advance of any bradycardia (13) and preceded signs of failing cardiovascular function (14). These hypoxic changes were found to reflect myocardial glycolysis and early hypoxic stress (6-12). The same changes were found in newborn babies immediately after birth (4) and were well correlated to the electrocardiographic pattern during labor and the clinical condition of the child after delivery (5). These

EKG recordings were registered with a bipolar precordial lead. In clinical practice, however, the FECG is registered mainly by the use of a vaginal electrode giving an unipolar scalp lead. The different ways of exploring myocardial electric activity might explain the controversy regarding the value of the FECG as an indicator of fetal well being during labor.

The aims of the present study were to compare the values of the unipolar fetal scalp lead and the bipolar precordial lead (CR lead) during graded hypoxia in lamb fetuses and to correlate the FECG-changes to blood gases, acid base status and cardiovascular functions.

## METHODS

The experiments were conducted on 18 ewes of mixed breed with 18 fetuses. Their gestational age was  $120 \pm 4$  days (mean value  $\pm$  SEM) and ranged from 94 to 140 days (term 147 days). The gestational age was either dated or estimated from fetal weight and crown rump length using standard curves (7). The fetal weight was  $1948 \pm 204$  g and ranged from 619 to 3370 g. The fetuses were acutely exteriorized. The anesthesia was induced with pentothal (50 mg/ml, 0.1 ml/kg) and was maintained with chloralose (25 mg/ml, 1.4 ml/kg). The anesthesia and preparation method have been described earlier by Rosen *et al.* (14).

The fetal ECG was recorded as a bipolar precordial lead (CR lead) with lead electrodes of a diameter of 0.8 cm placed subcutaneously. The exploring electrode was placed on the left part of the chest, between the apex and the left part of the sternum; the indifferent electrode on the right foreleg and the common ground on the right hindleg. In 15 fetuses the FECG was also recorded as an unipolar scalp lead with the electrode placed subcutaneously in the parietal region and the common ground connected to the metal table on which the fetus was placed and in 3 fetuses it was recorded as a bipolar scalp lead with electrodes in the scalp and precordium and the common ground connected to the metal table. The FECG impulses were recorded on a Grass polygraph 7 recorder.



## ECG score

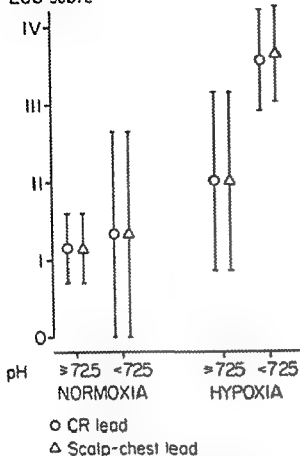


Fig. 5 The FECG score in relation to hypoxia and acidosis. Comparison between bipolar scalp-chest lead and CR lead (mean values  $\pm$  SEM).

always exceed that of the P wave. A scattergram of the T/QRS ratios is demonstrated in Fig. 3. The correlation coefficient between all the 74 observations is 0.51 ( $p < 0.001$ ).

**Bipolar scalp-chest lead.** The bipolar scalp-chest lead was used in 3 fetuses. Thirty-one sets of observations at varying  $pO_2$  and pH values were made. The material was divided in different groups in the same manner and mean values of  $pO_2$  and pH in the different groups are shown in Table II. The bipolar scalp-chest lead gave quite as good technical quality as the CR lead. It gave a good correlation to the precordial lead both as measured as T/QRS ratio with a correlation coefficient of 0.92 (Fig. 4) and as the scoring system with the highest scores in the group with both hypoxia and acidosis ( $3.55 \pm 0.65$  resp.  $3.64 \pm 0.62$  mean values  $\pm$  SEM) (Fig. 5).

## DISCUSSION

In an endeavour to detect fetal hypoxia, research has been focused on alterations in the fetal ECG diagram, but its diagnostic value is controversial. The present results are in accordance with our previous findings that the bipolar precordial FECG contains information regarding fetal hypoxia and acidosis both when the full configuration of the P period and when the T/QRS ratio is used as a criterion. The unipolar scalp lead, however, gave results which were more difficult to interpret. The heart rate was more often unstable, the QRS-complex was lower and the technical quality of the scalp ECG was not as good as the precordial lead. The bipolar system applied to the scalp lead did not correlate well with fetal hypoxia and acidosis as the CR lead. The T/QRS ratio of the scalp lead gave a somewhat better correlation with the fetal situation, the T/QRS ratios occurring when the fetuses were both hypoxic and acidotic. Since the QRS-complexes were generally small in the scalp lead, small elevations of the T wave gave higher T/QRS ratios but did not exceed that of the R wave. Thus the T/QRS ratio was inferior to the bipolar precordial lead in detecting fetal hypoxia and acidosis, yet it seems to be able to pick up ST-T changes using the scalp lead as exploring electrode. A bipolar scalp-chest lead was quite comparable with the precordial lead.

Alterations in the FECG have been reported during labor and with the aid of the scalp ECG and computer analysis when the FECG was found to be considerably improved (8, 11, 15). There are differences of opinion about the interpretation of alterations in the FECG during labor and there is no general consensus of opinion as to what are the changes in the configuration of the FECG are produced by changes in the fetal heart rate (1, 3, 7).

Table II pH and  $pO_2$  values in different groups (mean values  $\pm$  SEM) using the bipolar scalp-chest lead

	$pO_2$ (kPa)	pH
Normoxia without acidosis	$13.31 \pm 0.1$	7.38
Normoxia with acidosis	$13.10 \pm 0.19$	7.28
Hypoxia without acidosis	$11.16 \pm 0.11$	7.38
Hypoxia with acidosis	$10.61 \pm 0.11$	7.28

We have earlier shown in animal experiments that changes in the ST-T period of the FECG are an early sign of fetal hypoxia and that the same kind of ECG changes previously observed in animal fetuses exist already post partum in newborn human babies. The precordial bipolar CR lead was used both in the animal experiments and in the newborn babies. The present findings that the FECG registered with a unipolar scalp lead gives less information and is more difficult to interpret might explain the controversy regarding the value of alterations in the ECG during labor and asphyxia and it might be worthwhile trying some kind of bipolar scalp lead during labor in clinical conditions.

# ACKNOWLEDGEMENTS

This study was supported by grants from Göteborgs Läkaresällskap, the Faculty of Medicine, Gothenburg, the Swedish Medical Research Council (19 X 2591) and the Expressen Prenatal Research Foundation.

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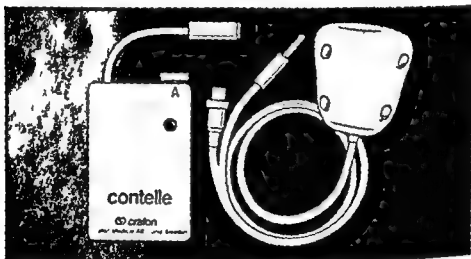
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Submitted for publication January 16 1979

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# RADIOIMMUNOASSAY OF SERUM BILE ACIDS IN NORMAL PREGNANCY AND IN RECURRENT CHOLESTASIS OF PREGNANCY

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## MATERIAL AND METHODS

**Abstract** Radioimmunoassay has been used to quantitate conjugates of cholic and chenodeoxycholic acid in serum blood from women in different stages of normal pregnancy and in patients with recurrent intrahepatic cholestasis of pregnancy (RCP). The levels of cholic and chenodeoxycholic acid were shown to be within normal limits throughout uncomplicated pregnancy and elevated in RCP. Because of RIAs simplicity and reliability it is suggested that this method can be used to detect and follow the course of RCP and to assess the possible influence of treatment e.g. diet.

Intrahepatic cholestasis of pregnancy (RCP) is characterized patho-physiologically by intrahepatic cholestasis of varying degree and elevated levels of bile acids in blood is one of the main features of the disease (8-14). Recently methods based on quantitation of individual bile acids by computerized GC/MS have made it possible to study changes of the urinary excretion of bile acids in great detail (1). The quantitatively important role of the primary bile acids, cholic and chenodeoxycholic acids, in RCP has been confirmed (16-17). The complexity of these methods never precludes their use in clinical routine work. Methods for determining conjugated bile acids in serum by radioimmunoassay (RIA) have been in use for more than 5 years and have proved to be sensitive and highly specific, simple and reliable (4-13). In order to evaluate the clinical use of bile acid determinations in pregnancy a study has been made in which the concentrations of cholic and chenodeoxycholic acid conjugates in serum measured by RIA have been compared with the excretion of these bile acids in urine determined by GC/MS.

**Normal subjects** Blood levels of conjugated cholic and chenodeoxycholic acid were determined 4 times during the last two trimesters of eight normal pregnancies. Venous specimens were taken in the morning after overnight fasting. All of the women subsequently had normal deliveries.

A second group of normals consisted of twenty-three pregnant women who were admitted to the delivery ward at term. Some of these were already in labor, others were admitted for induction. None of the subjects had any disease with possible liver engagement (e.g. toxæmia, diabetes) and all had been fasting for 8 hours before sampling. The deliveries were normal.

**RCP patients** Five patients with general itching and the clinical diagnosis of RCP were studied with analyses of conjugated cholic and chenodeoxycholic acid in blood (after overnight fasting), total excretion of bile acids in urine and conventional liver function tests once during the last pregnancy month (patients A, B, C, E, F). Apart from RCP the pregnancies and subsequent deliveries were normal. Two more patients (D and G) were followed during the last two trimesters at 4 week intervals with the same type of sampling. Details of urinary bile acid excretion in these two patients have been published (17).

Finally three women (patients H, I, K) with a history of general itching in previous pregnancies were followed during the two last trimesters with venous blood taken at monthly intervals. They all developed general itching after the 33rd pregnancy week. In these cases the samples were taken in the morning after a light breakfast.

**Radioimmunoassay** Blood was drawn into heparinized glass tubes from an antecubital vein and centrifuged. The plasma was stored at -20°C until analyzed. Unsulfated glycine and taurine conjugated cholic and chenodeoxycholic acid were determined according to Simmonds *et al.* (13). The method with a few minor alterations has been described (12). Crossreactions with other bile acids were negligible. The range in healthy fasting subjects is 0.1-1.0 µmol/l for cholic acid conjugates and 0.2-2.0 µmol/l for chenodeoxycholic acid conjugates.

**GC/MS analysis** Urine was collected during a 24-hour period and kept frozen during the collection period by storage on dry ice. After thawing and measuring aliquots were stored at -20°C until analyzed. Separation of the bile acids according to conjugate groups and computerized GC/MS quantitation of individual bile acids were achieved by the method of Alme *et al.* (1). Normal upper limits for cholic and chenodeoxycholic acid were 1.0 µmol/24 h and

Abbreviations and trivial names: cholic 3α,7α,12α-trihydroxy-5β-cholanoic acid and chenodeoxycholic 3α,7α-dihydroxy-5β-cholanoic acid. Recurrent intrahepatic cholestasis of pregnancy = abbreviated RCP. radioimmunoassay RIA. gas chromatography mass spectrometry GC/MS.

Table 1 Fasting serum levels ( $\mu\text{mol/l}$ ) of conjugated cholic (S-C) and chenodeoxycholic (S-CDC) acid in normal pregnancy

Pregnancy week	16-22		23-27		28-32		33-37	
	S-C	S-CDC	S-C	S-CDC	S-C	S-CDC	S-C	S-CDC
Subject								
1	0.48	2.15	0.16	0.41	0.15	0.70	0.4	1.7
2	0.10	0.20	0.14	0.62	0.40	0.46	0.28	1.1
3	0.22	1.60	0.83	3.70	0.70	2.13	0.1	1.1
4	0.70	0.31	0.35	1.15	0.52	1.60	0.38	1.1
5	0.60	1.25	0.34	0.65	0.28	0.77	0.90	1.1
6	0.16	0.22	0.23	0.75	0.70	0.57	0.50	1.1
7	0.27	1.15	0.20	0.88	0.21	0.80	0.28	1.1
8	0.35	1.40	0.20	0.37	0.27	0.85	0.36	1.1
Mean	0.36	1.04	0.31	1.07	0.34	0.98	0.4	1.1

See text

1.0  $\mu\text{mol}/24\text{ h}$  respectively (1, 16). No differences in these values were observed between healthy non pregnant and pregnant women.

**Routine liver tests** Serum aminotransferases (S-ASAT and S-ALAT) and serum alkaline phosphatases (S-ALP) were determined according to (13) and serum bilirubin (S-Bil) was measured with a Technicon multichannel analyzer.

## RESULTS

**Serum bile acids in normal pregnancy** Table 1 shows the result of serial determinations of cholic and chenodeoxycholic acid conjugates in the second half of eight normal pregnancies. All values except two are within the normal range. Subject 3 showed slightly increased levels of chenodeoxycholic acid on two occasions, probably because she had been breaking the fast before sampling. However, even on these occasions the levels of cholic acid were normal. Significant increases of the values towards the end of pregnancy were not observed.

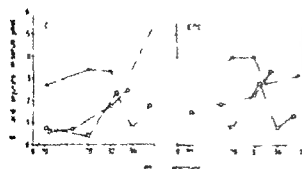


Fig. 1 Serum levels of conjugated cholic (C) and chenodeoxycholic (CDC) acid in three patients: H (x-x-x), I (o-o-o) and K (---).

The range of conjugated cholic acid in 27 women examined during delivery was 0.15-0.85  $\mu\text{mol/l}$  (mean 0.43  $\mu\text{mol/l}$ , SD 0.22  $\mu\text{mol/l}$ ). The corresponding chenodeoxycholic acid range was 0.1-1.7  $\mu\text{mol/l}$  (mean 0.86  $\mu\text{mol/l}$ , SD 0.37  $\mu\text{mol/l}$ ). **Serum and urinary bile acids in patients with cholestasis** The relationship between serum levels and excretion of cholic and chenodeoxycholic acid is seen in Table II.

Fig. 1 shows the serum levels of conjugated cholic and chenodeoxycholic acid in the last trimester of patients H, I and K. The values are close to normal in each of the patients. Thus, in these cases of cholestasis the cholic/chenodeoxycholic ratio in serum was relatively constant.

The levels of cholic and chenodeoxycholic acid in blood and the excretion of these bile acids from patients D and G are compared in Fig. 2. In cholestasis advanced and bile acid levels started to rise, the ratio of cholic to chenodeoxycholic acid increased. The same phenomenon was observed in urine.

**Routine liver tests** The levels of serum aminotransferases, alkaline phosphatases and bilirubin determined in the patients A-G are shown in Table II. There was no correlation between these values and the levels of serum cholic and chenodeoxycholic acid.

## DISCUSSION

Our study confirms earlier results that serum levels of conjugated cholic and chenodeoxycholic acid are normal in uncomplicated pregnancy (7, 14). The methods in previous studies were based on quantitation by gas chromatography.

Table II Primary bile acids in serum (S-C and S-CDC) and urine (U-C and U-CDC) and conventional liver function tests in ten patients with RCP. Normal values are shown within brackets. Serum bile acid levels in patients A-G are fasting levels; those in H-K are not.

Patient	Pregnancy Age week	S-C ( $<1.0 \mu\text{mol/l}$ )	S-CDC ( $<2.0 \mu\text{mol/l}$ )	U-C ( $<1.0 \text{ mmol/24h}$ )	U-CDC ( $<1.0 \text{ mmol/24h}$ )	S-ASAT ( $<0.7 \mu\text{kat/l}$ )	S-ALAT ( $<0.7 \mu\text{kat/l}$ )	S-ALP ( $<4 \text{ kat/l}$ )	S-Bil ( $<21 \mu\text{mol/l}$ )
22	35	54.0	12.0	170.1	58.5	1.45	3.06	13.2	38
30	40	35.0	10.0	100.2	57.4	1.38	3.00	11.1	19
23	33	16.0	12.0	32.5	30.5	2.16	3.70	14.6	15
28	38	12.5	7.2	9.3	5.2	2.87	7.64	9.1	5
27	40	12.0	8.0	4.6	6.8	0.51	0.71	11.6	9
24	36	2.3	2.2	2.3	3.5	0.67	1.28	2.7	10
31	37	1.7	1.1	4.1	3.0	0.63	1.14	6.3	7
29	40	5.5	3.1						
25	35	2.5	3.4						
27	39	1.8	1.4						

Normal value is normally elevated during pregnancy.

with the necessary steps of purification and hydrolysis makes these methods time-consuming and therefore impracticable in clinical use. It should be pointed out that with our RIA method the total amounts of glycine and taurine conjugates and about 50 per cent of the unconjugated bile acids are

determined but not the sulfated bile acids. However, since practically 100 per cent of cholic and about 90 per cent of chenodeoxycholic acid in serum are conjugated with glycine or taurine (10), omission of a time-consuming solvolysis seems justified. Also, in preliminary experiments, RIA determinations of cholic and chenodeoxycholic acid in serum were in good agreement with analyses by gas liquid chromatography after solvolysis.

After a test meal, cholic and chenodeoxycholic acid differ in absorption and hepatic turnover (2, 12). Cholic acid describes a small peak 60–90 minutes after the meal and a rapid decline. In contrast, serum levels of chenodeoxycholic acid increase more rapidly with a high peak after 30–60 minutes and decline slowly to fasting levels. Subject 3 in Table I illustrates this difference between the two acids. Probably because of variation in time between meals and sampling, chenodeoxycholic levels exceeded normal values on two occasions. Cholic acid apparently cleared more rapidly after meals, was within normal limits on both occasions. Therefore, RIA determinations of cholic acid conjugates in serum might be preferred as the routine method for serial analyses in e.g. recurrent cholestasis of pregnancy.

**Bile acid determinations in RCP.** Clinically, RCP patients can be divided into three groups: I, pregnant women with general itching only; II, patients with general itching and elevated serum aminotransferase levels; III, patients with general itching and elevation of serum aminotransferases as well as conjugated bilirubin (jaundice). Apart from the clinical assessment, the serum aminotransferases and especially alanine aminotransferase (S-ALAT) have been the best routine methods for following intermediate and

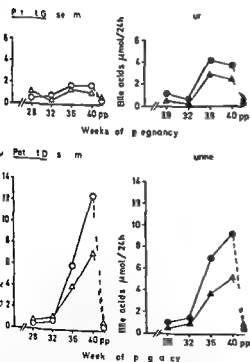


Figure 2 Conjugated cholic (O—O) and chenodeoxycholic (—Δ) acid in serum (after fasting) determined by RIA and cholic (□—□) and chenodeoxycholic (Δ—Δ) acid in urine determined by GC/MS in two patients D and G with RCP.

serious cases of RCP (5). Recent studies, however, have shown that there is a poor relationship between the levels of serum aminotransferases and changes in bile acid metabolism as judged by the excretion pattern of bile acids in urine (16). It seems reasonable to assume that the determinations of bile acid levels in either bile (9), serum (8), amniotic fluid (3) or urine (16) reflect the degree and course of RCP better than any method in use today. Table II and Fig 2 show the positive relationship between levels of conjugated cholic and chenodeoxycholic acid in blood and total urinary excretion of these bile acids. No such relations could be seen between any of the bile acids and the conventional liver function tests, except in the case of patient A, who had pathologically elevated serum levels of bilirubin.

Some studies indicate that RCP may represent a danger to the fetus (6, 11). A method for quantitative serial analyses of the degree of liver involvement in RCP would be of value in assessing the effect of treatment and in deciding whether or not labor should be induced. The simplicity and reliability of RIA determinations of bile acids in serum seem to make this method the best one at present for monitoring the course of RCP. It may also make it possible to study the effects of other complications of pregnancy, e.g. haemolysis, on the levels of bile acids in blood.

## ACKNOWLEDGEMENTS

The assistance of the Solna Antenatal Clinic staff is gratefully acknowledged.

This work was supported by grants from the Swedish Medical Research Council (grant number 3X 219 to Dr Jan Sjövall) and the Swedish Society of Medical Sciences.

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Submitted for publication February 14, 1979

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# OXYTOCINASE ACTIVITY IN THE COURSE OF CONTINUOUS LUMBAR EPIDURAL ANALGESIA

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**Abstract** The oxytocinase activity in the blood serum, placental blood and homogenates from the placenta and umbilical cord was determined in 34 women in labor subjected to continuous lumbar epidural analgesia with 0.5 per cent bupivacaine and 1:800 000 epinephrine. The results obtained were subjected to statistical analysis and compared with a group of 30 patients in spontaneous labor without any drugs. Significantly higher oxytocinase activity was found in the women subjected to epidural analgesia. Increased oxytocinase activity was found to correlate with the duration of the first stage of labor. It is assumed that the increase in the oxytocinase activity in the course of continuous epidural analgesia is associated with diminished uterine contractions and is related to the effect of the local anesthetic agent.

Oxytocinase (EC 3.4.11.3) the specific enzyme causing a loss of the biological properties of oxytocin is regarded as one of  $\alpha$  amine acyl hydrolases of peptidases. Immunohistochemical studies by Watkins & Hall (17) showed that oxytocinase which controls the oxytocin level of the blood is localized in the trophoblastic cells of the placenta and secreted into the intervillous spaces. According to Brandt & Ferner (3) this is accomplished through enzymatic inactivation of the neurohormone and its degradation in the tissues. The action of oxytocinase in processes related to maintenance of pregnancy initiation of labor in its course is beyond any doubt (8). Ances (1) has published observations concerning the behavior of oxytocinase activity in the course of spontaneous labor. The purpose of the present paper is to establish the behavior of oxytocinase in the course of labor with epidural analgesia. While the effect of the epidural analgesia on uterine contractility has been studied by several authors e.g. Cooper *et al* (5) and Ford (6) Schiffman (12) the pertinent literature fails to describe the mechanism involved.

## MATERIAL AND METHODS

At the Institute of Gynecology and Obstetrics Wrocław Medical Academy 34 primiparae were subjected to continuous lumbar epidural analgesia. Analgesia was induced with 0.125 per cent bupivacaine and 1:800 000 epinephrine at the onset of the active phase of labor (2). The lumbar epidural analgesia was carried out in all cases by Prof. dr hab. A. Aronowski, head of the Interclinic Department of Anesthesiology and Resuscitation Wrocław Medical Academy. The control group was made up of 30 primiparae with spontaneous labor and no drugs. There were differences as to age, clinical, laboratory and environmental data between the experimental and control groups.

The oxytocinase activity in the blood was determined on four occasions on each patient: before the onset of clinically detectable labor (A), at the active phase of labor (B), at full dilatation of the cervix (C), and two hours post partum (D). In both groups the oxytocinase activity was determined in the umbilical blood and in placental and umbilical homogenates.

The present method of determining the enzymatic activity is a modification of that reported by Tuppy & Nesvadba (15) and has been described earlier (19). The method is as

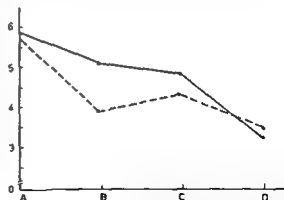


Fig. 1 Serum oxytocinase activity in different phases of labor: A Prior to labor, B Active phase of labor, C Full dilatation of the cervix, D Two hours post partum. — Epidural group, --- Control group. Oxytocin activity in IU/l.



Table I Oxytocinase activity in the blood serum and umbilical blood of women during spontaneous labor

Time of determination	Epidural group		Control Group		Test of significance $p < 0.001$
	Mean	SD	Mean	SD	
Prior to labor	5.81	1.51	5.69	1.94	—
Active phase of labor	5.12	1.41	3.94	0.81	+
Full dilatation of the cervix	4.89	1.50	4.30	1.69	+
Two hours post partum	3.17	1.44	3.50	1.59	+
Umbilical blood	1.53	0.70	1.07	0.33	+

Expressed in IU/l

ed on the use of L-cystine-di  $\beta$  naphthylamide from Koch Light Lab. England as a substrate and colorimetric determination of the  $\beta$  naphthylamine released with p-dimethyl aminobenzaldehyde in presence of acetic acid. Oxytocinase activity is expressed in IU/l. The duration of labor was also evaluated as were the contractility of the uterus and the fetal and placental weights: the results were subjected to statistical analysis.

## RESULTS

The results are given in Table I and Fig. 1.

Comparison of the oxytocinase activities before labor (A) in the epidural group ( $5.81 \pm 1.51$  IU/l) and control group ( $5.69 \pm 1.94$  IU/l) shows similar values the differences being insignificant. In the active phase of labor (B) the mean oxytocinase activity in the epidural group is  $5.12 \pm 1.41$  IU/l which is significantly higher than that in the control group with a mean value of  $3.94 \pm 0.81$  IU/l. In both groups the observed reduction in oxytocinase activity is related to the increase in the level of the circulating oxytocine which is lower in the epidural and higher in the control group and results in accelerated contractility of the uterus.

At full dilatation of the cervix (C) the mean oxytocinase activity in the epidural group amounts to  $4.89 \pm 1.50$  IU/l and is statistically higher than that in the control group with a mean  $4.30 \pm 1.69$  IU/l.

The determinations performed two hours post partum (D) were  $3.17 \pm 1.44$  IU/l in the epidural group and  $3.50 \pm 1.59$  IU/l in the control group: both groups showed a similar decreasing tendency (Fig. 1).

The oxytocinase values of the umbilical blood were inhibited significantly in both groups. In the epidural group the mean oxytocinase activity was  $1.53 \pm 0.70$  IU/l against  $1.07 \pm 0.33$  IU/l in the control. A significant increase of 50 per cent oxytocinase activity in the epidural group correlated well with the decrease in oxytocinase in the blood serum of pregnant women with analgesia.

The oxytocinase activity of placental homogenates as expressed in terms of IU/g of protein revealed no statistically significant differences, it was  $0.89 \pm 0.41$  IU/g in the epidural group and  $0.91 \pm 0.31$  IU/g in the control and correlated well with placental weight and fetal weight (Table II and III).

Analysis of the duration of individual stages of labor revealed a significant prolongation of the first stage in the epidural group amounting to  $713$  min against  $583$  min in the control. The difference was corroborated by a statistical analysis of the expression of a reduction in the contractility of the uterus related to the analgesia.

In both groups the differences between the duration of the second and third stages of labor were not significant (Table IV).

Table II Oxytocinase activity in homogenates from the placenta and umbilical cord

Site of determination	Epidural group		Control group		Test of significance $p < 0.001$
	Mean	SD	Mean	SD	
Placenta	0.89	0.41	0.91	0.31	—
Umbilical cord	0.76	0.23	0.70	0.38	—

Expressed in IU/g of protein

Table III Fetal and placental weights (g)

	Epidural group		Control group	
	Mean	SD	Mean	SD
Fetal weight	3 495.00	452.18	3 386.00	367.16
Placental weight	536.47	97.75	551.00	81.42
Significance				
0.001	+		+	

## DISCUSSION

In the control group the results of studies of oxytocinase activity in the course of labor are consistent with those observed in spontaneous labor by Ances who used a similar technique. The corresponding variations from the epidural group are different, particularly in the active phase of labor and at full dilation, since the average oxytocinase activity is higher than that in the controls. The increase in oxytocinase activity in the epidural group was also accompanied by temporary decrease in contractility of the uterus. This fact is evidenced by a mean prolongation of the first stage of labor of 130 min in the epidural group, which has also been observed by other authors (10, 11, 14, 18).

The concomitant increase in the oxytocinase activity in the blood serum of patients in the epidural group is suggested by the correspondingly higher oxytocinase activity (higher by 50 per cent) in the umbilical blood from the same group. The lack of differences in the oxytocinase activity of placental homogenates and blood serum in both groups implies that changes in oxytocinase activity during labor are closely related to the analgesia and agents used. Mack *et al.* (16) and Steenberg (13) when studying the contractility of the uterus in patients during the active phase of labor failed to find a correlation with bupivacaine concentration. The studies of Epstein *et al.* (9) revealed no correlation between the concentration of 1–2 per cent prilocaine and its blood level on the contractility of uterus. He agrees, however, with

Bromage (4) who states that there is little evidence to suggest that epinephrine in a concentration of 1:200 000 has a deleterious effect on labor if a low dose lumbar epidural technique is used. Nicholas *et al.* (9) demonstrated that 0.25 per cent bupivacaine with 1:400 000 epinephrine had no effect on the duration of uterine contractility.

Epinephrine is, however, known to cause a rapid and short lasting strong contraction of blood vessels in all organs. The changes in the oxytocinase activity of the blood serum and umbilical blood from the epidural group during labor on the one hand and the lack of such changes in the post partum period and in the homogenates on the other constitute further confirmation of the observation that epinephrine is almost immediately degraded by phenolase and aminooxidase.

Although the influence of various factors related to labor and the contractility of the uterus (e.g. progesterone, estrogen, nervous, anatomic or psychic effects) interferes with the evaluation of analgesic agents, the statistically established increase in oxytocinase activity seems to be one of the links in the chain of events leading to disturbed or diminished contractility of the uterus.

The present studies are a step towards a better understanding of complex and interdependent mechanisms which require further investigation.

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Table IV Duration (min) of stages of labor in epidural and control groups

P	First stage		Second stage		Third stage	
	Mean	SD	Mean	SD	Mean	SD
Epidural	713.16	534.08	56.32	40.57	6.50	5.48
Control	583.08	177.17	55.16	41.42	6.13	3.07
Significance						
0.001	+		-		-	

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Submitted for publication September 11 1977

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## ECTOPIC PREGNANCY AND IUDs INCIDENCE RISK RATE AND PREDISPOSING FACTORS

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During a period of 4 years 1974-77 in Uppsala county Sweden 93 women underwent surgery for ectopic pregnancy with histological proof of the diagnosis. For the whole population of fertile age this corresponds to 0.11 ectopics per 100 women 15-44 years of age or 1.08 per 100 live births or 1.53 per 100 births. Fifty five of the women with ectopic pregnancy were using an intrauterine device (IUD) (48 a copper bearing IUD and 7 some other type of device) and 6 women used a low dose progestogen contraceptive. For users of copper bearing IUDs the risk of ectopic pregnancy was estimated to be 0.15 per 100 women years. When comparing this latter risk rate with the national incidence rate of 0.11 it must be observed that the denominators forming the denominator in these two rates differ with respect to some crucial characteristics. Nulliparity and predisposing factors were found statistically significant more often in non IUD-users with an ectopic pregnancy than in IUD-users. Such predisposing factors may be less relevant in IUD-users as in other populations. This may explain why ectopic pregnancy has been found to occur less frequently than theoretically expected among IUD-users. The ectopic preventing capacity of the IUD may therefore be considerably lower than has been previously assumed.

The incidence of ectopic pregnancy is known to vary from population to population and also for users of different contraceptive methods including no contraception (7). Apart from obvious differences in the mode of action for different contraceptive methods the incidence might in part be explained by differences in the prevalence of conditions predisposing to ectopic pregnancy (1). In this paper we report findings on the prevalence of ectopic predisposing factors in women with ectopic pregnancy with and without an IUD. We present the incidence in a defined geographical area of Sweden and the estimated risk rate for ectopic pregnancy for users of copper bearing IUDs.

### MATERIAL AND METHODS

In registers kept in all the hospitals in Uppsala county Sweden every woman who had undergone surgery for ectopic pregnancy during the period 1974-77 was identified.

Their records were examined with respect to the history of previous pregnancies, infertility problems, tubal surgery, other abdominal surgery, pelvic inflammatory disease (PID) and the use of contraceptive method at the time of the ectopic pregnancy. The occurrence of tubal adhesions found during the operation was noted. The validity of the diagnosis of ectopic pregnancy was evaluated. Only those women who had histological proof of the diagnosis were included. All women were sent a questionnaire about the use of contraceptives at the time of their ectopic pregnancy and their history of PID prior to the operation.

Based on detailed sale statistics for copper-bearing IUDs in the county which we obtained from the wholesale firms we estimated the number of one year-users of this contraceptive method. The figures on sale statistics were reduced by 10 per cent to cover returns returned by retailers and IUDs discarded because of contamination. In addition the estimation takes into account the discontinuation rates per year. We have used the discontinuation rates for IUD-users given by Mishell (6) which are of the same magnitude as those found for users of copper bearing devices in a recent clinical trial in our department (9).

The size of the female population 15 to 44 years and the number of births and legal abortions were found in official demographic statistics (9, 10, 11). The number of spontaneous abortions was collected from registers in the hospitals of the county.

### RESULTS

During the years 1974 to 1977 a total of 203 women in Uppsala county underwent surgery for an ectopic pregnancy with histological proof of the diagnosis. All the patient records were found and examined. The questionnaire was answered by 97 per cent of the women.

Six ectopic pregnancies occurred among users of a low dose progestogen contraceptive. Fifty five women reported use of an IUD at the time of their ectopic pregnancy. Of these women 43 used a copper bearing IUD and 7 some other type of device. Among women using other contraceptive methods than oral contraceptives (OC) IUDs or no contraceptive at all (non-OC-or IUD-users) we registered 142 ectopic pregnancies. For the 6 women (3 per cent of

Table 1 Incidence of ectopic pregnancy Uppsala county 1974-77

Measure of incidence	Rate
Ectopics per 100 intrauterine pregnancies	1.08
Ectopics per 100 births	1.53
Ectopics per 100 women aged 15-44 years	0.11

the total number) who did not answer the questionnaire we obtained information on the use of OC or IUD from their medical records. The correlation between the answers to the questionnaire and the medical record information was high. We found contradictory information with regard to previous PID in only 3 of the women not using OCs or IUDs. According to their records two of these women had been hospitalized for PID. For the third woman we relied on her information from the questionnaire in which she denied previous PID.

The mean age for IUD users with an ectopic pregnancy was 27.8 years  $\pm$  5.2 and for non OC or IUD users 27.7 years  $\pm$  4.8.

From 1974 to 1977 the number of notified intrauterine pregnancies in the county was 18 773 (13 277 deliveries, 1 218 spontaneous abortions and 4 278 legal abortions). In Table 1 the incidence of ectopic pregnancy in the county during the observed period is presented. For users of copper bearing IUDs the risk of ectopic pregnancy was estimated to be 0.15 per 100 women years.

In Table II the recorded variables relating to the previous pregnancy and any history of events which may have interfered with fertility for both IUD users and for non OC or IUD users having an ectopic pregnancy are listed. (The small number of women using a low dose progestogen is excluded in this con-

text.) The table shows that the number of women with one or more predisposing factors was 13 (31 per cent) of the IUD users and 94 (55 per cent) of the non OC or IUD users ( $p < 0.001$ ).

During the laparotomy for ectopic pregnancy, fewer adhesions were recorded for IUD-users compared with non OC-or IUD users in 13 (31 per cent) of the IUD users and in 66 (46 per cent) of the non OC or IUD users. tubal adhesions were made, ( $p < 0.005$ ).

## DISCUSSION

Recently Aznar *et al* (1) have assessed the problems concerned in the evaluation of an association between ectopic pregnancy and the IUDs. They point out that the ratio of ectopic, intrauterine pregnancies which usually are used to describe the incidence of the former, is knowledge concerning the actual risk for use of these contraceptives. For IUD-users this ratio is affected by a contraceptive-dependent reduction in the intrauterine pregnancy rate. Therefore they suggest that the incidence of ectopic pregnancy should be expressed as life table rates per 100 years. They also stress that predisposing factors may vary between users of different contraceptive methods. This latter suggestion is probably not yet confirmed or rejected by careful case control studies. The prevalence of predisposing factors in women with ectopic pregnancies using different contraceptive methods would indicate whether this suggestion is of relevance.

In our county the incidence of ectopic pregnancy was found to be 0.11 per 100 women 15-44 years of age or 1.08 per 100 notified intrauterine pregnancies or 1.53 per 100 births. This incidence is lower

Table II Recorded variables in women with ectopic pregnancy by contraceptive method Uppsala county 1974-77

Variable	IUD users (n = 55)	Non OC-or IUD users (n = 14)	$\chi^2$ test	p-value
Previous birth	43	85	5.071	p < 0.05
Previous spontaneous abortions	6	15	0.035	NS
Previous legal abortions	6	18	0.009	NS
Previous PID	13	53	2.749	NS
History of infertility	1	41	15.774	p < 0.01
Previous tubal surgery	11	24	9.065	p < 0.01
Other abdominal surgery	8	34	1.565	NS
One or more predisposing factor	18	94	16.766	p < 0.01

$\chi^2$  values computed with continuity correction

ter than that found in Turku Finland for 4-75 (1.23 ectopics per 100 births or 83 per 100 pregnancies) (2) but somewhat lower than the incidence reported by Hallatt California USA for 4-75 (1.85 ectopics per 100 births) (3)

The estimated risk of ectopic pregnancy for users of copper bearing IUDs was 0.15 per 100 women years. Our estimated risk is therefore of the same magnitude as reported by Lehfeldt *et al* for a population in the USA (0.1 per 100 women years) (4) and Sey *et al* for a British population (0.12 per 100 women years) (8)

We find that the overall incidence of ectopic pregnancy for women in the fertile age span and the estimated risk for users of copper bearing IUDs within the same population are of the same magnitude. In comparison, these rates it must be observed that the denominator in the overall incidence rate comprises women of whom a large proportion are not at risk of conceiving (sexually inactive and sterile and pregnant women) or women at a very low risk (women using combined OCs) whereas the denominator in the risk rate for IUD users consists of sexually active women continuously at risk of conceiving. These differences in the population representing the denominator in our rates make them unsuitable for comparison.

Considering the women with ectopic pregnancy we had less nulliparous women and fewer women with a history of ectopic promoting factors in IUD users than in non OC or IUD users. These findings are in accordance with those reported by Hallatt (3) Erkkola & Luukko (2). They also found promoting factors occurring more often in non IUD users than in IUD users. Some of these factors such as infertility problems, history of salpingitis, Fallopian tube surgery for infertility and adhesions are certainly linked and more than one of these factors was tried for some of the women. Nevertheless in our study the proportion of IUD-users with an ectopic pregnancy without one or more predisposing factors was statistically significantly larger than the proportion of non-OC- or IUD-users with an ectopic pregnancy presenting with no such factor.

It has been claimed that in the presence of an IUD the implantation of a fertilized ovum is reduced by 95 per cent (4). This assumption is based on the difference between expected and observed number of ectopics in IUD-using women. Such a calculation does not take into account any factors such as a possible linkage between the choice of an IUD for

contraception and a low prevalence of ectopic promoting conditions. A low prevalence of conditions predisposing for ectopics in IUD users which our results suggest could explain why they occur less frequently than expected among users of this contraceptive method. The ectopic preventing capacity of an IUD may therefore be considerably lower than previously claimed. We find it reasonable therefore to avoid recommending IUDs as a method of contraception in women with known ectopic predisposing conditions.

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Submitted for publication January 3 1979

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## RESULTS IN THE OPERATIVE TREATMENT OF PELVIC ENDOMETRIOSIS

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**Abstract** Two hundred and eight patients treated surgically for endometriosis were reviewed one to four years later. The degree of endometriosis in this survey was 17 per cent of gynecological laparotomies. After operation 30 per cent of the patients were symptomless, 60 per cent estimated the condition as good or moderate, and only 10 per cent had benefit from the operation. In the 32 cases where infertility was the main indication for operation and other factors affecting fertility were excluded, 20 pregnancies of 19 men were achieved after operation.

The incidence of endometriosis is 15-25 per cent of gynecological laparotomies (3) and the frequency is said to be increasing with the trend towards smaller incisions (4, 5). In spite of new drugs, such as synthetic progestagens and danazol, surgery will obviously remain the principal means of treatment, especially in the case of a severe pain, infertility and adnexal mass.

In order to get information about the efficiency of the different operative methods, we analyzed retrospectively the patients operated on for endometriosis in our hospital during the years 1971-76.

## METHOD AND PATIENTS

During the study period, the incidence of endometriosis was 17 per cent of all laparotomies or scopes performed in the gynecological department of our hospital. Two hundred and eight patients were available for a follow-up study one to four years later (mean interval 2.6 years). One hundred and eighty-seven patients attended the hospital clinic and were interviewed and examined by one of us. Twenty-one patients returned a filled-in questionnaire. The age of the patients at the time of the surgery ranged from 20 to 53 years (Table I). Half of the patients were over 35 years. Low parity was associated with endometriosis. Forty-nine per cent were nulligravidae or had one child.

The three cardinal symptoms before surgery were dysmenorrhea, lower back pain and dyspareunia (Table II). The other common complaints included pain on walking, rectal pain, nausea, infertility and menstrual disorders.

On pelvic examination, adnexal mass and tenderness were present in 57 per cent of the cases, nodularity and tenderness of the pouch of Douglas and uterosacral ligaments in half of the patients, and fixed retroverted uterus in 25 per cent.

Eighty-eight patients had been treated with hormones (synthetic progestagens or estrogen-progestagen combination) before surgery. In these cases, 8 per cent had experienced the response as good, while 50 per cent had had no relief.

Table I A Distribution of patients according to age

	<25	25-29	30-34	35-39	≥40
no.	12	36	42	42	83
per cent	6	17	20	20	40

Table I B Distribution according to parity

Parity	0	1	2	3	≥4
no.	56	46	47	33	26
per cent	27	22	22	16	13

Table II Percentage of different symptoms 208 patients

	Per cent
Dysmenorrhea	92
severe	76
moderate	16
Low back pain	82
severe	59
moderate	23
Dyspareunia	59
severe	31
moderate	28
Pain on walking	40
Rectal pain	37
Nausea	29
Infertility	29
Menorrhagia	28
Diarrhea	22
Dysuria	17
Fever	17



Table III Distribution of operations

	No	Per cent
<b>Radical</b>		
Hysterectomy + bilateral salpingo-oophorectomy	51	25
Hysterectomy + preservation of some ovarian tissue	33	16
Total	84	41
<b>Conservative</b>	124	59
Resection + presacral neurectomy	51	25
Resection	45	22
Electrocoagulation of endometrial implants	4	2
Laparoscopy	19	10
Total	124	59

As assessed during the operation the disease most frequently involved simultaneously the ovaries, the pouch of Douglas and the uterosacral ligaments (42 per cent). In one third of the patients only the ovaries were affected.

**Operative therapy.** Fifteen patients had been previously operated on for endometriosis. Total abdominal hysterectomy and bilateral salpingo-oophorectomy with excision of the areas of endometriosis was performed on 51 patients (Table III). In 33 cases using the same procedure some ovarian tissue on the other side was preserved. Fifty-one patients had conservative surgery which included excision of the areas of endometriosis, resection of the ovaries and the uterosacral ligaments and presacral neurectomy with suspension of the uterus. Forty-five patients had only resection without neurectomy.

Of the patients whose age exceeded 39 years, 82 per cent had radical surgery. For those under 30 only conservative operations were performed.

## RESULTS

Thirty per cent of the patients were symptomless after operation. 41 per cent estimated the overall result as good and 19 per cent as moderate. Only 10 per cent experienced deterioration or had no help from the operation. Radical surgery resulted in 99 per cent relief of the presenting symptom. In the conservative surgery group with neurectomy 90 per cent of the patients had complete or some relief of the actual symptoms. When only resection was made a moderate or good result was achieved in 80 per cent of the cases. Thus neurectomy improved the result by 10 per cent. Dysmenorrhea was present in 191 women before operation and 84 per cent of them had complete or moderate relief of the symptom. Lower back pain and dyspareunia were affected in the same manner (Table IV). In 15–24 per cent of the cases the actual

symptom was unchanged or aggravated after operation.

Eighty patients had a trial on progestagen therapy after operation (mean duration of treatment 4 months). This did not improve the results.

During the follow up time 17 patients (11 per cent) had a recurrence of the disease that demanded reoperation. They all had had conservative surgery. Infertility was the main indication for reoperation in 60 cases. In spite of that 87 per cent had radical surgery because the disease was so extensive. The rest of the patients, 43, had conservative resection or only laparoscopy (9 cases). Concerning the location of the endometriosis this group did not differ from the other patients. After exclusion of all other factors affecting fertility (deficient male factor 9 and use of contraceptives 2) radical surgery was followed by laparoscopy (17), a 63 per cent pregnancy rate was achieved in 32 patients, 9 of these 32 women had had preoperative or postoperative progestagen therapy, but they had both preoperative and postoperative progestagen therapy, pregnancy failed in only 2 cases. The rest of the patients had either preoperative or postoperative progestagen therapy, they had 6 pregnancies. The mean interval from the operation to conception was 10 months (range 4–24 months) and it was not affected by hormonal therapy. These 20 pregnancies of 19 children ended in normal delivery in 17 cases, in early abortion in 2 cases and in one legal interruption of pregnancy.

## COMMENT

The reported incidence of endometriosis estimated in pelvic explorations varies from 15 to 25 per cent (3) though an incidence as high as 40 per cent

Table IV Effect of the operative therapy on different symptoms of endometriosis. The results are given in percentages in parentheses the number of patients with the symptom before operation.

Symptom	Complete relief	Modest relief	No relief
Dysmenorrhea (191)	41	43	16
Low back pain (171)	44	41	15
Dyspareunia (123)	53	29	18
Pain on walking (93)	54	26	20
Rectal pain (77)	54	26	20
Nausea (60)	53	33	14
Diarrhea (46)	49	31	20
Dysuria (33)	51	31	18

and recently in a large series by Williams (5). In the present survey the incidence was 17 per cent. Age, parity, presenting symptoms, clinical findings and the location of endometriosis in this study are in accordance with the results reported earlier (3). Symptom relief after operation was good in our patients. Similar results have been reported by Garcia and Williams (5). The best results were achieved with radical surgery. When conservative surgery was employed, presacral neurectomy added some 10 per cent to the good results. With hormonal pseudopregnancy treatment, satisfactory results have been reported in 84–98 per cent of cases (4). In our survey, only half of the patients had satisfactory results from hormonal therapy. The disease may have been more extensive in these patients because they were later selected for operative therapy. Andrews (1) reports as high as 68 per cent recurrence of the signs or symptoms of the disease after pseudopregnancy. In our study the pregnancy rate was 63 per cent and slight improvement was noted when the patient had not been treated with synthetic progestagens both pre- and postoperatively. In the series of Andrews (1) a 9 per cent pregnancy rate was reported after surgery and 8 per cent after pseudopregnancy. A combination of the two treatments resulted in an even better pregnancy rate in Andrews' series. Our results

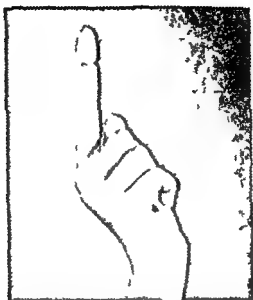
confirm that surgery still remains the principal therapeutic means for pelvic endometriosis. This is especially true when severe pain, adnexal mass or infertility is present.

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*Submitted for publication March 28, 1979*

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# URETHRAL PRESSURE PROFILE AT PUBOCOCCYGEAL REPAIR FOR STRESS INCONTINENCE

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## MATERIAL AND METHODS

**Abstract** The influence of pubococcygeal repair on the urethral pressure profile as well as the cure rate, technical results and side-effects have been studied in 16 severely stress incontinent patients. The patients were followed during the first postoperative year in order to study the possible long term effects of devascularization, traction and scarring on the urethral tone. Examination using simultaneous urethrocystometry with recording of the urethral pressure profile in rest was undertaken preoperatively, 3 months postoperatively and one year postoperatively. Urethral functional length was not affected by surgery. Urethral closure pressure was slightly reduced 3 months postoperatively and did not recover completely in the first postoperative year. Such a reduction in urethral pressure probably reflects damage to the urethral muscle due to devascularization and denervation. Its main clinical importance concerns cases of severe stress incontinence with a very low initial closure pressure in the urethra. Here vaginal surgery may jeopardize the success of otherwise technically unapproachably performed operation. On the whole, pubococcygeal repair is effective in restoring continence although the side effects call for careful selection of patients.

Pubococcygeal repair (4) results in a cure rate of approximately 85 per cent in severely stress incontinent women (7). We know from earlier studies that the restoration of continence is not greatly dependent on improvement in urethral tone or pressure at rest, even though efficient support for the urethra has been established (5). The advantages of good support of the urethra are only revealed in a stress situation, not at rest (3). Unfortunately the surgical procedure which involves extensive dissection beneath the bladder and the urethra, often with abundant bleeding, may damage urethral vascularization and traction, thus reducing tone in the urethral wall as well as causing and scarring continue postoperatively. We have studied the influence of pubococcygeal repair on the urethral pressure during the first postoperative year by means of simultaneous urethrocystometry with recording of the urethral pressure profile at rest.

**Operative technique** An extensive arcuate incision in the infratonsus is used to get good access to the urethra and the posterior aspect of the bladder (Figs 1 and 2). Extensive ligation of vessels in this area may be necessary. The so called bladder ligaments are sutured together in the midline, elevating the bladder neck sufficiently to obtain continence on provocation, such as a cough if the patient is under epidural anesthesia or direct pressure suprapubically if general anesthesia is used (Fig. 3). Then both pubococcygeal muscles are cut and their anterior portions are sutured together under the bladder neck and the proximal urethra, creating a muscle sling loosely arranged which just prevents excessive downward rotation of the urethra on stress (Figs 4 and 5). The posterior parts of the muscles are attached to the ischio-cavernosus muscles laterally. Finally the bulbocavernosus muscles are partly freed and sutured together as a support for the distal urethra without division (Fig. 6). Excessive parts of the anterior vaginal wall may be resected and the intratonsus incision is closed. A vaginal pack is needed as well as a suprapubic catheter for urinary drainage. A coexisting rectocele may or may not be treated at the same time. Levator sutures will be somewhat less efficient, since the pubococcygeal parts of the levators have been cut for the creation of the muscle sling. The pack is removed after 24 hours and the catheter can usually be removed after 5-8 days.

**Subjects** Sixteen women, mean age 57 years (range 31-77) and mean parity 2.3 (range 0-4), suffering from clinically verified severe stress incontinence, who had urinary leakage on moderate exertion such as laughing, walking up stairs, rising and running. No urgency was present and urinary cultures were sterile. Cystoscopy was normal. No medical treatment had been tried except estrogens a couple of weeks preoperatively in all cases. This treatment was discontinued immediately postoperatively. Some patients had tried pelvic muscle exercise with poor result.

**Method** Simultaneous urethrocystometry with recording of the urethral pressure profile was performed preoperatively (before estrogen medication) as well as 3 months and 1 year postoperatively. The diagnosis of pure severe stress incontinence without complicating factors had been established and the patients were operated on as described above. Postoperatively continence was checked and side-effects noted. The position of the bladder neck, i.e. the technical result of the operation was evaluated. Residual urinary volume was checked at all examinations.

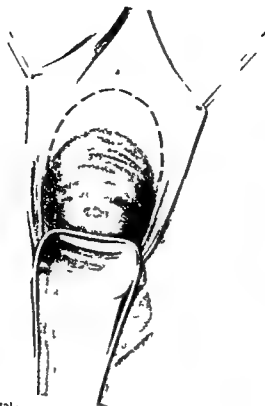


Fig 1 The midline incision

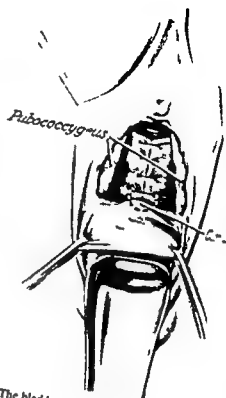


Fig 3 The bladder ligaments sutured in the end

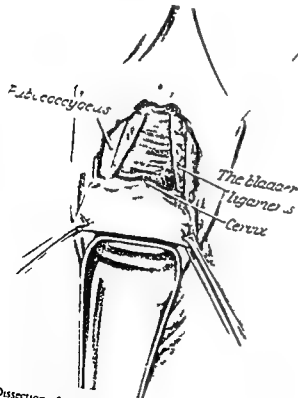


Fig 2 Dissection of the anterior vaginal wall and exposure of the bladder ligaments and the pubococcygeal muscles

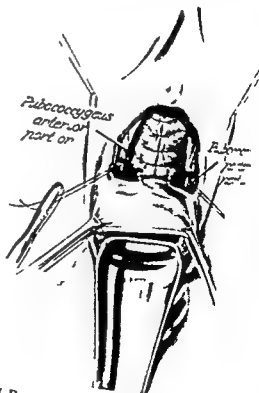


Fig 4 Division of the pubococcygeal muscles

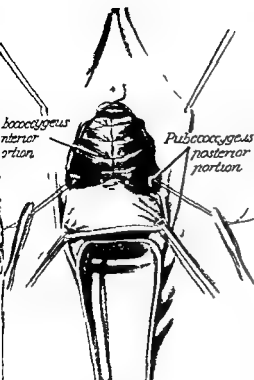


Fig 5 The formation of a muscle sling by suturing the anterior portions of the pubococcygeal muscles together

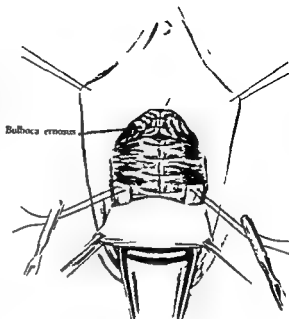


Fig 6 The bulbocavernosus muscles sutured together beneath the distal urethra

The equipment for simultaneous urethrocystometry has been described by Asmussen & Ulmsten (1, 2). A thin catheter contains two sensor areas 6 cm apart. Via transducers and amplifiers the urethral pressure, the bladder pressure and the difference between them, the urethral closure pressure, can be recorded. The catheter is inserted through the urethra by an electric motor at constant speed until the proximal sensor has passed the entire urethra. The distal sensor remains in the bladder. Thus the urethral pressure profile, i.e. the pressure registered continuously from the internal to the external opening of the urethra, is recorded. The parameters of the urethral pressure profile are the functional length, the maximum urethral pressure and the maximum closure pressure (Fig 7). The patients were examined in the lithotomy position. The bladder was filled with saline solution at 25 ml/min through a baby feeding catheter. After each 100 ml three urethral pressure profiles were recorded, the third one being used for analysis (6). The patients were also asked to hold their urine to test their ability to voluntarily improve urethral closure. Cystometry was continued until the bladder capacity was reached or until the bladder contained 500 ml.

## RESULTS

From Table I there is a marked tendency towards a lower closure pressure 3 months postoperative

ly. However, a statistically significant difference is not a constant finding at all bladder volumes. At 300 ml bladder content there was no statistically significant difference between pre- and postoperative values; otherwise the difference was clear ( $p < 0.05$ ). One year postoperatively there was still a tendency although less marked, with statistically significant differences only at 200 and 400 ml bladder volume ( $p < 0.05$ ). The functional length of the urethra was unaffected by surgery.

Holding urine resulted in an increase in closure pressure in half of the patients preoperatively and in half of the patients postoperatively, although not necessarily the same ones.

Three months postoperatively 15 patients were cured and one improved. The bladder neck was well elevated in all cases and downward rotation on straining was limited. One year postoperatively 14 patients were still cured, one had a slight degree of stress incontinence and one was as severely stress incontinent as before the operation. The bladder neck was still well elevated in all cases.

Two women had a persistent residual urinary volume of more than 50 ml. Only 5 women were free

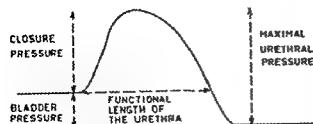


Fig 7 The urethral pressure profile and its parameters

from side-effects or recurrence. Major side-effects were protruding rectocele and hypertrophy of the cervix. The side effects are listed in Table II.

### DISCUSSION

It was apparent that pubococcygeal repair did not dramatically alter the shape of the urethral pressure profile at rest, which is in accordance with our earlier findings (5). The newly established support for the urethra and the bladder neck did not increase either the functional length or the closure pressure at rest. On the contrary, there was a strong tendency towards a lowered closure pressure 3 months postoperatively. It is likely that the dissection of the anterior vaginal wall with the extensive ligation of vessels which is often necessary, may damage urethral tissues and innervation enough to partly destroy urethral tone, thereby reducing urethral pressure. In stress incontinence, any further loss of closure pressure is undesirable, especially if the initial closure pressure is already

Table I Urethral closure pressure and functional length before and after pubococcygeal repair for stress incontinence ( $n = 16$ )

Bladder volume ml	100	200	300	400	500
Mean urethral closure pressure mm Hg					
Before surgery	25.1 (9.65)	4.8 (7.98)	1.9 (9.34)	4.4 (8.30)	20.3 (6.20)
3 months after surgery	18.9 (6.78)	17.5 (7.3)	17.1 (7.2)	17.7 (7.31)	14.3 (6.20)
1 year after surgery	22.0 (5.40)	18.8 (5.71)	19.3 (6.86)	17.4 (6.17)	16.5 (6.6)
Mean urethral functional length mm					
Before surgery	3.3 (4.6)	1.1 (3.1)	0.3 (5.0)	20.1 (4.5)	19.4 (2.9)
3 months after surgery	3.1 (1.9)	3 (3.3)	1.3 (3.4)	20.4 (4.1)	18.7 (3.4)
1 year after surgery	3.8 (3.1)	2 (4.5)	9 (5.1)	9 (4.8)	3 (5.8)

Standard deviation in parenthesis  $n = 10$   $n = 15$   $n = 15$

Table II The side-effects after pubococcygeal repair in the 16 women examined

Side-effect	No.
Rectocele	4
Cervical hypertrophy	3
Intestinal stenosis	1
Urgency	1
Urethral stenosis	1
Coital disturbances	5
Residual volume ( $> 50$ ml)	7
Great loss of closure pressure	1

out of 10 sexually active, 1 leading to recurrence

ready low. Therefore, if alternative operative techniques are available, it may be wise not to operate vaginally in cases with a very low preoperative pressure. Otherwise, reduction in closure pressure would reduce the usually good result of the operation. Thus, in spite of a well-elevated bladder neck, the patient may be in stress incontinence as she was before beginning. This is what happened to the one patient in our study who, one year postoperatively, had improved. However, the suggestion that the operation may result in progressive scarring, thereby destroying urethral tone, could be discarded, as further reduction in closure pressure was seen in the immediate postoperative period.

In other respects, the results agree well with earlier experience that pubococcygeal repair is effective in achieving continence, the muscle strengthening contractile in half of the patients. The side effects are multiple. Some degree of rectocele postoperatively is almost unavoidable as a consequence of the rearrangement of the levator muscles. A prolapso or colpocephaly does not prevent the later occurrence of rectocele (7). Besides, it is difficult to create a stable posterior vaginal wall since the levator is and of little use as support. It is possible that rectocele may be of benefit urodynamically, acting as a bulging support under the urethra and bladder neck during stress. We believe that it should be left untreated provided it does not impair function.

Cervical elongation and hypertrophy may occur as a consequence of the bladder being fixed to the cervical stroma, promoting the tendency to be drawn towards the introitus as the uterus shrinks.

The disorganisation of the vaginal wall after pubococcygeal repair may cause coital problems.

na may have become too wide the introitus too low sensitivity impaired or intercourse may be painful because of roughen scarred surfaces. Accordingly it may be wise to use pubococcygeal repair with caution in young and sexually active women.

### CONCLUSION

Pubococcygeal repair tends to impair urethral tone. Reduction in tone may be of importance clinically. The initial urethral closure pressure at rest is low preoperatively. Otherwise the possible side effects on the urethral pressure are more compensated by the achieved improvement in pressure transmission to the urethra (3).

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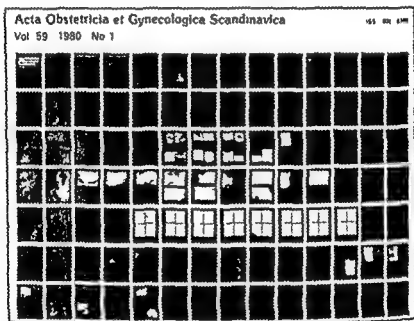
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*Submitted for publication December 12, 1978*

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## RESPONSE OF ENDOMETRIOID OVARIAN CARCINOMA IN NUDE MICE TO THE COMBINATION OF VINCISTINE SULPHATE AND CYCLOPHOSPHAMIDE

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The effect of a combination treatment with vincristine sulphate and cyclophosphamide to endometrioid carcinoma grown in nude mice hosts was studied by pathological, ultrastructural and biochemical methods. The first course of treatment had little or no effect. After second and third courses, however, the growth of the tumor was suppressed, as evidenced by increased necrosis and decreased weight of tumors. The total volume of the tumor decreased, but there was no change in the nuclear-cytoplasmic ratio and other ultrastructural features. The DNA and RNA contents showed a decreasing trend.

Complete remission was observed during the treatment in two treated animals kept for a longer observation period. The tumors regressed completely and no tumor growths were found. In the control animals, the tumor grew progressively and the histology was identical to that of the primary tumor. However, the frequency of mitoses was higher in the transplanted tumor than in the primary tumor.

The nude mouse model for the testing of the sensitivity of human carcinomas to various antineoplastic agents is potentially of great value. The human endometrioid carcinoma system fulfills a number of criteria for a useful model in cancer chemotherapeutic studies. The most important advantage is that a human cancer can be treated in a living organism in the same way as in a cancer patient and it is possible to study simultaneously the correlation between the toxicity of the treatment and the benefit in the whole organism. In these respects, the nude mouse model is superior to any *in vitro* model. A variety of human malignant tumors can be transplanted to nude mice (11). The characteristics of the human donor material are well preserved (9).

Endometrioid ovarian cancer is often radiosensitive and intrapelvic for a relatively long period (6). Local recurrences and extrapelvic processes are often difficult to control. Like endometrial carcinoma, it generally shows a relatively poor response

to cytostatics. However, our clinical observations (5) indicate that these tumors in certain cases respond well to the combination of vincristine sulphate and cyclophosphamide. Therefore we undertook a study of the effects of this combination *in vivo* in nude mice hosts by histopathological, ultrastructural and biochemical methods.

## MATERIAL AND METHODS

100 seven-week-old SPF female nude mice nu/nu BALB/c/A/BOM (GL Biohologard Ltd, Denmark) were used for the studies. The animals were kept under strict SPF conditions in Makrolon® cages at  $27 \pm 1^\circ \text{C}$  and a humidity of  $55 \pm 5$  per cent. Autoclaved pellet food (Astra EVOS) and pasteurized water were provided *ad libitum*.

The tumor specimen of poorly differentiated endometrioid carcinoma of the ovary was obtained at laparotomy. A solid graft which appeared to be active cancer tissue was chosen. The tumor tissue was transferred in sterile test tubes containing Dubcos essential medium without antibiotics. The tissue tubes were kept at  $4^\circ \text{C}$  for less than one hour. The tumor tissue was divided into approximately 1 mm cubes. One hundred test animals were inoculated subcutaneously with the solid tumor tissue in the right and left flanks. After 35 days the treatment was started with 78 successfully transplanted mice.

The animals were divided into sixteen experimental groups. All test animals received 0.015 mg/kg vincristine sulphate (Oncovin® Lilly) and four hours later 10 mg/kg cyclophosphamide (Läike) intraperitoneally. On the second and the third days the cyclophosphamide treatment was repeated and on the fourth day the test animals were given both vincristine and cyclophosphamide. Four test groups received second and third courses one and three weeks after the first treatment. The control groups received saline at the same time.

The weight of the mice and the size of the tumor were determined twice weekly (Fig 3). In the test groups and in the respective controls the samples for examination were taken 4, 7, 10 and 13 days after the treatment. The animals were decapitated and the tumors removed. The weight and the size were determined before a piece from each tumor was cut and fixed in neutral formalin, embedded in paraffin and



Fig 1 Nude mouse 30 days after subcutaneous inoculation with 2 mm cubes of human endometroid ovarian carcinoma in the right and left flanks

stained with van Gieson and Hematoxylin Eosin for histological examination. Multiple samples from each tumor of the animals decapitated after the third course were taken and processed for electron microscopy as described by Kleini and Grönroos (6). In addition the change in the nucleocytoplasmic ratio and the total mitochondrial volume of the tumor cells were calculated. This was done by measuring the areas of the nuclei, cytoplasm and mitochondria in the electronmicrographs with an image analyser (MOB 3 Konttron Meschgeräte GMBH). The rest of the tumor was frozen on CO<sub>2</sub> ice for determination of the DNA and RNA contents. The statistical analyses were carried out using Student's *t* test.



Fig 2 Human endometroid ovarian carcinoma grown subcutaneously in a nude mouse host in the control group 30 days after inoculation with a 2 mm cube of tumour tissue

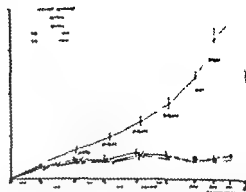


Fig 3 Differences in relative tumour growth in nude mice treated with three vincristine sulphate, cyclophosphamide courses during different periods

## RESULTS

The tumors grew fast and had attained a remarkable size 30 days after inoculation (Fig 1). Twenty per cent of the animals were eliminated from the experiment because of unsatisfactory growth of the tumor. A very low rate of spontaneous mortality was observed during the experiment. Four animals died in the control groups and two in the treated groups.

In the control groups the size of the tumors increased during the experiment to such an extent that animal's movements were hampered (Fig 1). Therefore the animals of the last control group were sacrificed 10 days before the last treated group.

As shown in Fig 3 the relative growth of the tumors increased progressively in the control group.



Fig 4 Human endometroid ovarian carcinoma removed from a laparotomy H & E  $\times 229$



Human endometrioid ovarian carcinoma grown subcutaneously in a nude mouse for 35 days after inoculation  $\times 260$



Fig 6 Human endometrioid ovarian carcinoma grown subcutaneously in a nude mouse 14 days after the end of treatment with three courses of combined vincristine sulphate-cyclophosphamide H & E  $\times 184$

growth stopped in the treated groups during the 1st course of treatment and the tumor's size remained almost constant to the end of the experiment. Differences between relative tumor growth in the treated and untreated groups were statistically significant after the second course of treatment.

The histology of the primary tumors of the patient and nude mice was similar, with a slightly higher frequency of mitosis in the latter. The tumor consisted of solid nests of cells and of glands in loose connective tissue. The epithelial cells of the tumor were polygonal in shape and size and contained large nuclei, many of which were in mitosis (Fig 4 and 5).

The effect of the combination therapy was analysed histologically after the first and third courses. A third course did not affect the tumor. The tumors of the animals decapitated four days after the third course consisted of solid areas, gland and hemorrhagic cyst formations surrounded by necrotic tumor tissue. Mitotic figures could still be seen in abundance.

The tumors of the animals decapitated on the 10th day after treatment showed similar histology, but the necrotic areas were larger. In the groups decapitated 10 and 14 days after the treatment, three quarters of the tumor tissue were necrotic. The viable part consisted of small glandular areas and mitotic figures could still be seen (Fig 6). No complete remission was observed during the 14 days after treatment, even in two animals which were followed up for 1 month after treatment: a complete regression of the necrotized tumor was found. After this the animals showed no signs of new tumor growth.

At the ultrastructural level the cancer cells were elongated or round with irregular and deeply infolded nuclei. The cytoplasm contained many mitochondria, some profiles of rough endoplasmic reticulum, primary and secondary lysosomes and some free ribosomes.



Fig 7 Low magnification electron micrograph of the endometrioid ovarian carcinoma before treatment. At the top of the figure there is seen a lumen of a gland (asterisk). One cell is in mitosis (m). Nuclei contain one or two nucleoli. In the cytoplasm there are many mitochondria, free ribosomes and some profiles of rough endoplasmic reticulum  $\times 6240$

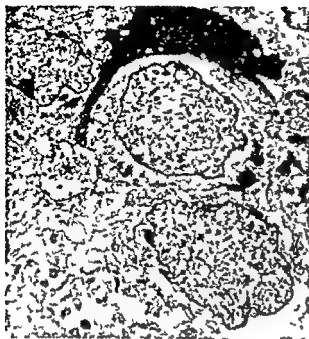


Fig 8 Low magnification electron micrograph of the endometrioid ovarian carcinoma after treatments. The tumor still forms glands (asterix) and also otherwise ultrastructurally resembles the untreated one  $\times 6240$

somes and polysomes. The free surface of the cells was sometimes covered with tubby microvilli and occasionally a cilia was observed. Compared with the controls the total volume of the mitochondria decreased ( $p < 0.001$ ) but there was no changes in the nucleo-cytoplasmic ratio and other ultrastructural features after the third course (Figs 7 and 8).

The DNA and RNA contents correlated with the macroscopic and microscopic findings. The content had a tendency to decrease after treatment. The RNA content in mg/g dry weight decreased from  $8.5 \pm 1.8$  (S.E.) to  $5.6 \pm 1.2$  in the tumors of the test animals decapitated four days after the third course and correspondingly the DNA contents from  $45.8 \pm 3.2$  to  $38.6 \pm 4.9$ . The differences are not statistically significant; they can only be considered indicative as no determinations were made in the 14-day group.

### DISCUSSION

The results obtained in this study confirm previous reports of successful transplantations of human malignant neoplasms into nude mice (10, 8, 3). In the present study we showed that transplanted endometrioid ovarian carcinoma is viable in nude mice hosts.

The karyotype of the neoplasms was preserved after transplantation but with a slightly higher frequency of mitoses which has also been reported by Gryn and Detre and Gazet (2). This may be due to the fact that the transplanted tumor grafts were more and therefore better vascularized than the original tumor.

In the modern oncologic treatment more and more giving place to the use of selective combined agents. Both endometrial carcinoma and endometrioid ovarian carcinoma have in clinical practice in certain cases shown a good response to a combination of vincristine sulphate and cyclophosphamide. This was true also in this experimental study. The therapy was intended to mimic clinical models as far as possible (7, 4) but with shorter intervals to suite the faster metabolism of mice. The results between the different courses in our preliminary experiments may however have been too high. Despite cessation of tumor growth some endometrioid carcinoma tissue could still be seen 14 days after the third course of treatment. From the morphological point of view the changes in the amount of nuclei and in the volume of mitochondria were good markers for determining the effect of the treatment.

This study supports the usefulness of the tumor/nude mice model in selective screening of anticancer agents or their combinations for activity against this kind of tumors. The method may also be useful for testing new treatment modalities. In studies of cancer tissue immunological studies, tumor cell kinetic research and studies of the mechanism of action of antineoplastic agents. The experience from this study suggests that in routine use the time for the entire procedure could be shortened to approximately one half.

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*Submitted for publication February 21, 1979*

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# RADIOIMMUNOASSAY OF hCG AS AN EARLY DIAGNOSIS OF CEREBRAL METASTASES IN CHORIOCARCINOMA PATIENTS

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**Abstract** To determine the early cerebral involvement of choriocarcinoma the following studies were employed. By measuring hCG titers in measurements of serum and cerebrospinal fluid (CSF) hCG- $\beta$  through radioimmunoassay (RIA) serum/CSF ratios of titers were calculated in patients with normal pregnancies and in those with trophoblastic neoplasia. In normal pregnancies the mean ratio of serum/CSF hCG was more than  $45.5 \pm 6.0$  (mean  $\pm$  SEM). In neoplasia without metastases to the brain the ratio was more than  $41.0 \pm 1.1$ . However 2 patients with cerebral choriocarcinoma showed low ratios of  $23.0 \pm 1.1$  and  $20.9 \pm 1.1$  respectively. This suggests that determination of hCG concentration ratio of serum to CSF using RIA might give a more reliable evaluation for the early detection of choriocarcinoma metastasized to the brain.

and blood from patients were obtained simultaneously by lumbar puncture and antecubital vein puncture respectively. Both CSF and blood were stored at  $-20^\circ\text{C}$  after immediate centrifugation.

HCG RIA was done as follows. Purified hCG  $\beta$  subunit from Serono Immunochemicals (Italy) was labelled with ( $^{125}\text{I}$ ) from the New England Nuclear Corp. (Boston, Ma, USA) by the enzymatic method (1). Specific antisera to the hCG  $\beta$  subunit were provided by the National Institute of Arthritis and Metabolic Diseases (USA). Purified hCG (Serono) served as the reference preparation for the assays which were carried out by the double antibody technique described by Vaitukaitis (7). The intra assay variance coefficient was  $\pm 8$  per cent and the inter assay coefficient  $\pm 15$  per cent. The assay showed a sensitivity of 10 mIU per milliliter.

## RESULTS

**Normal pregnancies** HCG concentrations of normally pregnant women were assayed in serum and CSF throughout gestation. The mean ratio of hCG titer in serum to that in CSF in the second month of pregnancy was  $128.5 \pm 13.2$  (mean  $\pm$  SEM), representing the highest ratio in all trimesters. After this highest value the mean ratio began decreasing to  $98.6 \pm 1.5$  in the third month of pregnancy and to  $52.8 \pm 4.0$  in the fourth month.

Because some CSF samples (1 of 5 in the seventh month of pregnancy and 2 of 5 in the ninth month) had undetectable values making the ratio impossible to calculate the mean ratios were greater than  $45.5 \pm 6.1$  in the seventh month (Table I, Fig 1).

**Molar pregnancies** HCG concentrations in 8 of 9 patients with hydatidiform mole showed ratios ranging from  $265.6 \pm 1$  to  $433.8 \pm 1$  while a patient with partial mole showed a ratio of  $41.0 \pm 1$  (Table II).

Although 3 of 8 patients followed up after evacuation of hydatidiform mole showed hCG ratios of  $44.8 \pm 1$ ,  $46.0 \pm 1$  and  $50.0 \pm 1$  respectively the rest had undetectable CSF hCG concentrations. All of these 8 continued a benign post molar course.

## MATERIAL AND METHODS

For hCG assay were collected from 9 patients with a hydatidiform mole 8 during treatment after evacuation of hydatidiform mole 4 with destructive mole 5 with choriocarcinoma and 77 normally pregnant women. CSF



Table I Normal pregnancies

Gestational age	No of subjects	Ratio of hCG concentration Serum (M±SE) CFS
II	5	128.5±13.2 1
III	8	93.6±1.5 1
IV	14	52.8±4.0 1
V	11	57.7±5.7 1
VI	3	62.0±12.4 1
VII	5	>45.5±6.0 1
VIII	6	59.1±10.1 1
IX	5	>48.2±7.4 1
X	20	54.5±3.6 1

Table II hCG concentration (mIU/ml)

hCG concentration (mIU/ml)	Ratio Serum CFS
<b>Hydatidiform mole</b>	
1 1 333 200 4 380	304.1
2 1 000 000 2 400	415.1
3 800 000 1 800	444.1
4 2 435 000 6 300	345.1
5 1 960 000 4 300	453.1
6 950 000 2 400	395.1
7 425 000 1 600	365.1
8 1 000 000 2 700	3 04.1
9 41 000 11 000	41.0
<b>Post molar pregnancy</b>	
1 1 000 UD	
2 33 600 750	44.1
3 330 UD	
4 1 500 UD	
5 9 600 UD	
6 2 300 50	46.0
7 UD UD	
8 1 200 24	50.0
<b>Destructive mole</b>	
1 13 020 240	92.1
2 7 750 140	53.1
3 34 100 110	310.0
4 62 300 360	173.1
<b>Choriocarcinoma</b>	
1 39 200 380	103.2
2 70 400 120	586.7
3 1 255 000 5 000	251.0
4 5 300 230	23.0
5 9 600 460	20.9

\* UD = undetectable

**Destructive moles** Four patients diagnosed histologically as destructive mole *in utero* without metastatic lesion showed hCG ratios ranging from 52.1 to 310.0. These patients entered complete remission following chemotherapy and hysterectomy.

**Choriocarcinoma** Of 5 patients with choriocarcinoma 3 with no evidence of cerebral metastasis—despite electroencephalography, cerebral angiography and radionuclide brain scan—showed hCG ratios of 103.2, 586.7 and 251.0 respectively.

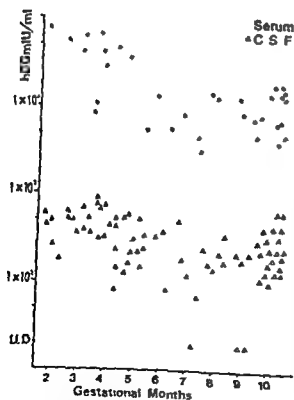


Fig. 1 Serum/CSF hCG levels in normal pregnancy

Two patients suspected of having cerebral metastasis showed hCG ratios of 23.0 and 20.9 respectively.

**Experiment of hCG transfer from CSF to blood** Theoretically, if hCG molecules transfer from one compartment to another, comparing hCG concentration in one with that in another is not useful for evaluating the hCG production.

To ascertain whether or not a blood-CSF barrier exists in the pathway of hCG from CSF to blood, we designed the following experiment. A female dog weighing 11.5 kg was maintained under anesthesia using ketamine with a pressure transducer (gauge No. 21) set between C11 and C12. A cannula inserted continuously CSF dropper (40 000 U/ml) suspended with 2 ml of saline was inserted into the femoral vein and 0.5 ml of CSF was collected every 15 minutes until 3 hours later.

When hCG traces were not detected in any CSF samples a further experiment was developed as follows. One ml of a solution containing 2 300 000 u/ml of  $^{125}\text{I}$  hCG  $\beta$  subunit suspended with a buffer was injected into the CSF. 0.3 ml of CSF collected minutes later still contained 186 652 cpm/0.1 ml of  $^{125}\text{I}$  hCG. 0.3 ml of CSF collected 3 hours later contained 49 000 cpm/0.1 ml of  $^{125}\text{I}$  hCG and 0.2 ml of CSF collected 4 hours later contained 26 600 cpm/0.1 ml of  $^{125}\text{I}$  hCG. No isotope content was detected however in 2 ml of blood collected every 15 minutes until 4 hours later.

## DISCUSSION

Organs involved in 512 cases of choriocarcinoma reviewed by Park & Lee (3) were as follows: 94 per cent vagina, 44 per cent brain, 28 per cent liver, 28 per cent kidney, 25 per cent and 21 per cent. Vaughan & Howard (8) reported that 70 per cent of their chorioepithelioma cases had brain metastases. Of 92 trophoblastic tumor cases in the Department of Obstetrics and Gynecology, Mayo Medical College, pulmonary metastases were found in 55.4 per cent and brain metastases in 13 per cent (5). It is particularly difficult to detect early brain metastases of choriocarcinoma before neurological signs and cerebral involvement develop even though diagnostic methods such as radioactive brain imaging, cerebral angiography and electroencephalography have often been employed. McCormick (2) measured gonadotropin titers in cerebrospinal fluid using a biological assay of hCG in cases of trophoblastic disease. By infusing a high concentration of hCG into a nephrectomized dog, he found that the ratio of gonadotropin value in blood to daily urinary output and to spinal fluid was approximately 1:374. Tashima *et al.* (6) noted that the CSF titer of hCG was markedly elevated in confirmed cerebral metastases from choriocarcinoma, but they concluded that hCG did not appear in CSF until a high threshold value—equivalent to a urinary excretion rate of about 250 000 IU of hCG/day—had been exceeded. Rushworth *et al.* (4) measured hCG concentrations in plasma and CSF by RIA in 19 cases of trophoblastic disease and found that the plasma:CSF ratios were above 100:1 in most patients with brain metastases and below 35:1 in patients without intracranial metastases. They also proved that

hCG concentrations in CSF relative to plasma were especially high in patients whose active tumors were located mainly in the brain or spinal cord.

Our study using homologous RIA of  $^{125}\text{I}$  hCG  $\beta$  subunit/anti hCG  $\beta$  subunit demonstrated that a ratio of hCG concentration in serum was correlated to that in CSF from the first to the third trimesters of normal pregnancies.

Discrepancies in ratios observed in a gestational month might be attributable to (a) changes in spinal fluid volume, (b) changes in spinal fluid pressure or (c) changes in permeability.

Our experiment with the dog suggested that a blood-brain barrier exists between the two compartments of blood circulation and CSF, since the content of hCG infused into circulating blood was not detected in CSF until 3 hours later, and  $^{125}\text{I}$  hCG  $\beta$  subunit infused into spinal fluid had still not transferred to peripheral blood 4 hours later. Thus, due to the apparent existence of a blood-brain barrier, metastases to the CNS may be diagnosed by calculating the ratio of hCG concentration in serum to that in CSF.

In 77 normally pregnant women and in 26 patients with trophoblastic disease, serum:CSF ratios ranged above 30:1, except for 2 patients with brain metastases who showed ratios of 23:1 and 20.9:1 respectively. It therefore seems probable that the critical ratio involving cerebral metastases is below 30:1 in choriocarcinoma patients.

Measurement of hCG in CSF may not be necessary to confirm the presence of intracranial metastases, and then lumbar puncture may be hazardous if increased intracranial pressure is present. However, occult cerebral metastases are often present without evidence of CNS lesion, and therefore the method we have indicated of detecting metastases at an early stage is needed.

As mentioned above, we should like to emphasize that evaluation of the hCG concentration in a serum:CSF ratio is useful for confirming the existence of choriocarcinoma in the CNS.

## ACKNOWLEDGEMENTS

This study was supported by cancer research grants from the Ministry of Health and Welfare, Japan, and from the Tokyo Medical College Cancer Center.

We wish to thank the American National Pituitary Agency through the Hormone Distribution Office, U.S. National Institute of Arthritis and Metabolic Diseases, for providing specific antisera to the hCG  $\beta$  subunit.

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*Submitted for publication January 1 1979*

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## ENDOMETRIAL EFFECT OF ORAL ESTRIOL TREATMENT IN POSTMENOPAUSAL WOMEN

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**Abstract.** Estriol is a weak estrogen with a claimed specific action on the epithelium in cervix uteri and vagina and with or limited ability to induce endometrial proliferation. In previous pharmacokinetic study we have shown elevated blood levels for only 2-3 hours after oral administration of the drug. The present study was performed to test if estriol when given orally in daily divided doses to postmenopausal women has any effect on the endometrium. Twenty postmenopausal women who had no vaginal bleeding in response to lynestrenol (Orgamettol<sup>®</sup>) 5 mg  $\times$  2  $\times$  V were treated with 6 mg estriol a day (Ovesteron<sup>®</sup>) 2 mg  $\times$  3 divided into three doses for 2 up to 3.5 months. When lynestrol (3 mg  $\times$  2  $\times$  V) was given following the estriol treatment periods 12 women out of 20 experienced a vaginal bleeding and one reported spotting. Endometrial biopsies in all of those women who had a bleeding were examined histologically. The endometrium was atrophic in four women, proliferative in two, slightly hyperplastic in one and showed signs of weak hormonal activity in one. Two women had a retrograde endometrium. It is concluded that estriol administered in a way that gives prolonged elevation of the blood levels is able to produce the same effect on the endometrium as other estrogens.

The present study was performed to examine if estriol when administered orally in daily divided doses had any effect on the endometrium in postmenopausal women.

### MATERIAL AND METHODS

Postmenopausal women scheduled for surgical treatment of uterine prolapse were enrolled for the study. None of them had been on estrogen therapy during the last year. To exclude women with a proliferative endometrium, lynestrenol (Orgamettol<sup>®</sup>) 5 mg was given twice a day for five days. The effective oral dose of lynestrenol in the Kaufmann test in climacteric women is 35-70 mg/cycle (12). Eleven women who got a vaginal bleeding after lynestrenol treatment were excluded from the study. These women were generally younger than the average for the group studied. Twenty women with no withdrawal bleeding were recruited for the study. They were 53 to 76 years old and had had their last vaginal bleeding at least one year ago. All 20 women got 6 mg estriol (1 mg tablets of Ovesteron<sup>®</sup>, Organon) a day orally for a minimum of two and a maximum of 3.5 months. Estriol was given in three doses daily (2 mg  $\times$  3). Immediately after the treatment period all but one woman got lynestrenol in the same way as before estriol treatment started. One woman did not complete the study.

Dilatation and curettage were performed in 10 out of 12 women who got a withdrawal bleeding after the lynestrenol treatment at the end of the estriol treatment. Two women did not collaborate. Specimens from the endometria were examined histologically at the Department of Pathology of University Hospital, Uppsala.

### RESULTS

**Treatment periods and results in each individual are listed in Table I.** None of the women had a withdrawal bleeding when lynestrenol was given before the estriol treatment. During treatment with estriol three out of 20 women had spotting after two months. Spotting was defined as a minimal vaginal bleeding demanding a minimum of sanitary protection.

When lynestrenol was given immediately after the estriol treatment period 12 women had a vaginal bleeding, one had spotting.

The relationship between estrogen replacement therapy and endometrial cancer has stimulated interest in estriol for the treatment of postmenopausal estrogen deficiency symptoms (1, 10, 15, 19) with its claim of weak or no effects on the endometrium and its acribally specific action on the epithelium of the cervix and vagina (2, 8).

The rapid conjugation of orally administered estriol explains the short biological effect after a single administration (14). Despite this, estriol is reported to reverse vasomotor symptoms without inducing endometrial proliferation (18). Estriol is known to bind to human endometrium (17) but a rapid decline in the clear receptor-estriol complex has been described after a single dose (3). With repetitive hormone injections to rats the potency of estriol is similar to that of radiolabeled estriol with respect to the induction of uterine growth (3).

Age years	Lynestrenol 5mg 2 "	E 1 or 2 mg 3			Lynestrenol 5 mg 2 "	DEC PAD
	Bleeding	Period of treatment			Bleeding	Endometrium
		1	2	3 months		
76	—	—	—	—	—	atrophy
68	—	—	—	—	—	desquamation secretion
66	—	—	—	—	—	
65	—	—	—	—	—	slight hyperplasia
64	—	—	—	—	+	proliferation
62	—	—	—	—	—	
62	—	—	—	—	—	atrophy
61	—	—	—	—	—	
61	—	—	—	—	+	secretion
60	—	—	—	—	+	
60	—	—	—	—	+	atrophy
58	—	—	—	—	+	
57	—	—	—	—	+	
57	—	—	—	—	+	atrophy
57	—	—	—	—	+	
54	—	—	—	—	+	
54	—	—	—	—	+	proliferation
53	—	—	—	—	+	weak hormonal activity

Fig 1 Age distribution of subjects treatment periods with lynestrenol (Orgametil<sup>®</sup>) and estradiol (Ovestin<sup>®</sup>) in twenty postmenopausal women. The second lynestrenol treatment period started immediately after the estradiol treatment period. No vaginal bleeding is indicated by — vaginal bleeding by +. Results of the histological examination of the endometrium in those women who got a vaginal bleeding are given to the right in the figure.

Histological examination showed an atrophic endometrium in four women. The endometrium was proliferative in two women and slightly hyperplastic in one. Secretory in one and secretory and desquamating in one.

One woman with vaginal bleeding and one with spotting did not accept curettage.

## DISCUSSION

In the present series of patients the estrogenic effect of estradiol on the endometrium was clear. The same endometrial changes as induced by estradiol were found.

Estradiol is a short acting estrogen. In a recently completed pharmacokinetic study we have shown that women receiving 1 mg and 12 mg of estradiol orally in a single dose showed elevated plasma levels of estradiol already after 15 minutes with peak concentrations after 45 minutes. In most women the concentration was down to pretreatment levels after 3 hours (5). Recently Schiff *et al* reported that administration of estradiol either vaginally or orally resulted in a significant decrease of serum FSH and LH levels (14). In Schiff's study peak levels of unconjugated estradiol were measured one hour after vaginal administration.

The estradiol concentration then declined. Oral administered estradiol was rapidly conjugated. There was however some elevation of urinary estradiol 30 minutes after and as late as 4 hours following the oral dose.

The nuclear binding time of estradiol has been studied in rats (3, 16). The binding time is short and only early uterotrophic events are observed. True uterine growth. To achieve late uterotrophic events a minimum of 6 hours of receptor occupancy is required (3, 16).

A likely explanation for the estrogenic potency of estradiol reported in this study is that when estradiol is administered orally in repetitive daily doses, the estradiol levels will be elevated for a sustained period allowing the nuclear receptor to be occupied long enough to stimulate late uterotrophic events and cellular proliferation.

The classification of estradiol as a weak estrogen is probably valid when estradiol is administered orally. Tzingounis *et al* (18) administered once a day for six months to postmenopausal women. They found clinical effectiveness on vasomotor stability and vaginal epithelium but failed to show induction of endometrial proliferation. The lack of sensitivity of the endometrium may reflect the schedule of administration or low doses used.

Puck observed that estradiol given at 5 mg a day for 5 days (a total dose of 30 mg) to 5 menopausal women did not induce withdrawal bleeding and there was no microscopic evidence of endometrial stimulation.

Haskins *et al* treated 60 women with 1 mg estradiol a day for 28 days. The patients ranged in age from 59 to 94 years. Three patients showed evidence of withdrawal bleeding following therapy. The ages were 48, 70 and 72 years and they were noted to have thickening of the vaginal mucosa at a level greater than the mean of the study group. Post withdrawal bleeding occurred in one of the patients who received megestrol on completion of the estradiol regimen. In two patients the endometrium was examined histologically. Minimal estrogenic effect was noted (6).

Recently Myhre reported the outcome of estradiol treatment in 213 postmenopausal women (17). The dosage varied from 1 mg/day up to 8 mg/day. The treatment was suspended for three days. The observation time was between 1 and 3.5 years. No bleeding was reported when the estradiol dose was 2 mg or lower.

on 3 mg estrinol per day experienced bleeding stage revealed an atrophic endometrium in all

user *et al* (7) found an estrogenic effect on the metrium in 39 of 40 postmenopausal women estrinol succinate was administered orally The was 2, 4 and 8 mg daily for at least 14 and up days The degree of stimulation of the endomet was not a linear function of increased dosage uration of treatment However they did not re whether estrinol was administered once or several daily

most studies mentioned above estrinol has been nistered once a day and/or in small doses Our show that daily repetitive oral administration of is able to induce estrogenic activity in the en etrium as estradiol

rol has been claimed to be a noncarcinogenic even an anticarcinogenic estrogen (9) and has classified as a safe estrogen When single doses ven estrinol competes with estradiol for nuclear ng sites but is dissociated from the receptor be late uterotrophic events are stimulated (4) When nistered in daily repetitive doses resulting in sus d elevated estrinol levels the receptor estrinol com n occupy nuclear retention sites equally well as e prior estradiol complexes late uterotrophic is are maximally stimulated and there is no an nism (4)

conclusion the mode of estrinol administration is factor that determines whether or not the drug have an estrogenic effect on the end organs Our indicate that estrinol is able to produce the same as on the endometrium as other estrogens if it is nistered repetitively resulting in prolonged elev i of the blood levels and continuous stimulation e estrogen receptors

## ACKNOWLEDGEMENTS

study was supported by the Swedish Medical Research vti (Grant No. 0349) the Ford Foundation and mon Oss

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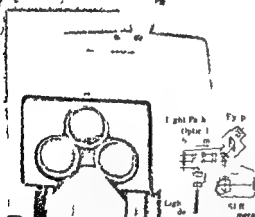
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Submitted for publication March 13 1979

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# THE INFLUENCE OF ADRENALECTOMY ON THE BIOMECHANICAL PROPERTIES OF COLLAGENOUS STRUCTURES OF RATS IN THE POST PARTUM PHASE

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Pregnancy influences the functional properties of genital and extragenital connective tissues. The sex hormones induce a major part of these changes but also corticosteroids may play a role as their concentration is elevated during pregnancy and early post partum. The mechanical properties of the pubic symphysis, muscle and skin of intact and adrenalectomized rats during the first ten days of the post partum phase were investigated. For the pubic symphysis and the muscle tendons of intact animals there was a decrease of the stiffness in the early post partum phase followed by a restoration. For adrenalectomized animals there was no such initial decrease which indicates that relaxation during pregnancy is less pronounced. However the strength of muscle and skin from the adrenalectomized animals was markedly decreased during the post partum phase. The stiffness of skin from the intact animals was increased in the early post partum period. This increase was also found in adrenalectomized animals but here the strength and stiffness were not only restored to the intact level but became even increased during the post partum period. It seems therefore that the adrenal glands not only participate in the regulation of adaptive changes during pregnancy but also play a role in the stabilization of extragenital connective tissues in the post partum phase.

connective tissues of the female genital tract are influenced both morphologically and functionally during pregnancy (5, 12). Extragenital collagenous tissues are also influenced during pregnancy with respect to their mechanical properties (12). The tensile strength of the pubic symphysis as well as muscle tendons in the female rat was thus found to be decreased at the end of pregnancy and during the post partum period. The mechanical contraction of the collagen fiber bundles in muscle tendons was increased during the post partum period (12). The mechanism behind these extragenital changes in the collagenous framework is unclear but sex hormones probably play a major role. The mentioned change in the pubic symphysis can be elicited by a combination of estrogen and progesterone (3, 6). However the role of corticosteroids in

these reactions should also be investigated as their concentration is elevated during pregnancy and the first 3-5 days post partum (1).

Connective tissues with high metabolic activity are found in the genital tract during pregnancy and post partum as well as in granulation tissues. Robertson and Sanborn (11) measured the amount of collagen produced in carrageenan granulomas after treatment with and deprivation of various hormones. Estrogen treatment as well as adrenalectomy increased the amount of collagen produced in the granulomas while cortisone treatment reduced it. Conversely Jørgensen (7) found no effect associated with adrenalectomy on granulation tissue formation nor on the content of collagen and hexosamines. His findings are in agreement with those of Chassin *et al.* (2) that adrenalectomy did not influence the healing of abdominal incisional wounds during the ten first days. The reports on the effects of adrenalectomy are thus conflicting.

The present series of experiments was undertaken to study the influence of adrenal corticosteroids on the restorative processes in the connective tissue outside the genital tract during the post partum phase. For this purpose the biomechanical properties of extragenital connective tissues from intact and adrenalectomized rats during the first ten days of the post partum phase was investigated.

## MATERIAL AND METHODS

Fifty five Wistar rats divided into groups according to Table I and 150-170 days old when sacrificed were used. The animals were mated when in the estrus phase and the day of parturition was recorded. All animals were housed under constant conditions with regard to room temperature, air humidity and light and had access to food and water *ad libitum*. 0.9 per cent sodium chloride was added to the water given to the adrenalectomized rats. Adrenalectomy was performed 3 weeks prior to the mating and thus 6-7 weeks before



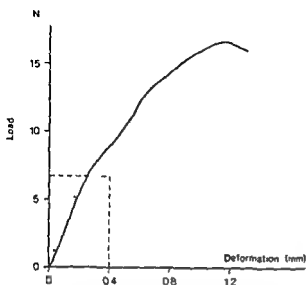


Fig 1 A typical load deformation curve for the pubic symphysis. The beginning and end of the linear region is indicated. The part of the diagram as in Fig 2 is marked by broken lines

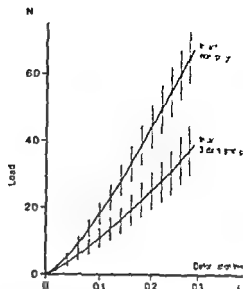


Fig 2 The first part of the mean load-deformation for the pubic symphysis from non-pregnant and 3-day postpartum intact animals. SEM indicated by bars

fore sacrifice. The adrenal glands were excised through incisions in the paravertebral musculature. The adrenalectomy technique was checked by determining plasma corticosteroids in three adrenalectomized rats which were injected with corticotropin 10 IU one hour before blood sampling and sacrifice.

After sacrifice by means of either overdosage, inspection and microscopic examination confirmed that the adrenal gland sites did not contain any glandular tissue. The dorsal skin caudal to the scapular region was removed including the subcutaneous muscle after the hair had been cut close to the skin. Standardized 2 mm wide strip specimens were cut at right angles to the longitudinal axis of the animal using a dice with two parallel mounted razor blades. The muscle tendons were left in situ in the exarticulated hind limbs

under intact skin. The tendon of the peroneus longus was dissected out immediately before testing. The bone and skin specimens were mounted into two sets of clamps with the free distance between them being 11 mm. During testing the tendon specimens were immersed in Ringer's solution (buffered to pH 7.4) at room temperature.

The coracal bones were dissected free and the skin consisting of the pubic symphysis and the two test bones was wrapped in saline moistened gauze and stored for more than one hour when not tested immediately. The bones were mounted into clamps which were shaped for the external surfaces of the bones. Each specimen was fixed against each clamp with a band of polyethylene tubing.

Table I Body weights (mean value  $\pm$  SEM)

Group	Number	Weight gram
Intact		
Non pregnant	8	248 $\pm$ 13
Post partum		
3 days	7	275 $\pm$ 13
5 days	6	300 $\pm$ 16
10 days	7	293 $\pm$ 11
Adrenalectomized		
Non pregnant	6	255 $\pm$ 15
Post partum		
3 days	6	297 $\pm$ 22*
5 days	7	280 $\pm$ 30*
10 days	8	289 $\pm$ 24*

\*  $p < 0.05$  against intact non-pregnant

†  $p < 0.05$  against adrenalectomized non-pregnant

Table II Biomechanical properties of the pubic symphysis (mean  $\pm$  SEM)

Group	$\Delta L_{max}$ (mm)	$F_{max}$ (N)	$\Delta L/F_{max}$ (mm/N)
Intact			
Non pregnant	1.18 $\pm$ 0.14	15.6 $\pm$ 1.9	0.075
Post partum			
3 days	1.76 $\pm$ 0.34	14.0 $\pm$ 0.5	0.125
5 days	1.78 $\pm$ 0.29	17.5 $\pm$ 0.8	0.101
10 days	0.93 $\pm$ 0.36	13.3 $\pm$ 0.6	0.070
Adrenalectomized			
Non pregnant	1.44 $\pm$ 0.14	13.3 $\pm$ 1.0	0.108
Post partum			
3 days	1.5 $\pm$ 0.23	14.0 $\pm$ 0.6	0.107
5 days	1.49 $\pm$ 0.08	14.1 $\pm$ 0.4	0.106
10 days	1.46 $\pm$ 0.19	12.4 $\pm$ 0.9	0.117

\*  $p < 0.05$  against intact non-pregnant

Table III Amount of collagen per unit specimen length (mean value  $\pm$  SEM)

group	No	Muscle tendon mg/mm	Skin mg/mm
Intact			
Non pregnant	8	0.108 $\pm$ 0.008	0.431 $\pm$ 0.016
Partum			
3 days	7	0.122 $\pm$ 0.009	0.437 $\pm$ 0.030
6 days	6	0.124 $\pm$ 0.006	0.427 $\pm$ 0.026
9 days	7	0.107 $\pm$ 0.010	0.449 $\pm$ 0.032
Adrenalectomized			
Non pregnant	6	0.129 $\pm$ 0.015	0.463 $\pm$ 0.021
Partum			
3 days	6	0.144 $\pm$ 0.006	0.433 $\pm$ 0.015
6 days	7	0.125 $\pm$ 0.011	0.501 $\pm$ 0.033
9 days	7	0.143 $\pm$ 0.005	0.491 $\pm$ 0.020

The materials testing machine (Aiwetron T 250) was used at a constant deformation speed of 2 mm/min and the specimens tested until failure. The load and deformation sensors were coupled to measuring bridges (Lorentzen Jette) and the signals recorded as a continuous X-Y trace on a Hewlett Packard Model 7004 recorder. For the pubic symphysis load-deformation data were read into a Hewlett Packard 9830A calculator. Mean curves and the  $F_{max}$  were calculated. The stiffness was estimated as the tangent of the angle between the linear region of the curve and the X-axis ( $\tan \alpha$ ). These data for the symphysis were further transformed as it is geometrically as well as mechanically complex. A typical load-deformation curve of the pubic symphysis is shown in Fig 1. For the curves from skin and tendon specimens coordinates for every strain interval of 0.01 were read and the load-deformation data transformed to stress-strain. Stress values were calculated from load data using amount of collagen per unit specimen length as functional cross section area.  $\sigma_{max}$  is maximum stress value, i.e. the ultimate tensile strength. The deformation values were expressed in units of original length, i.e. strain.  $\epsilon_{max}$  is the strain value at point of maximum stress. The stress-strain curve plotted out from these transformed data on Hewlett Packard 9862A as shown in Figs 3 and 4. The collagen contents of the tendon and skin specimens were calculated according to Neuman & Logan (8) from the hydroxyproline content determined according to the method described by Grant (4) on a Technicon auto-analyser. For each group mean values and standard errors of the mean for the different parameters were calculated on the basis of the mean values for the tests on each animal in the group. The F-max test (13) was employed to verify the homogeneity of variances and the t-test according to Student Newman-Keuls was used to test the differences between the groups. Differences were considered significant if  $p < 0.05$ .

## RESULTS

The pregnancies came to full term in all animals without complications. The fetuses from the adrenalectomized animals were generally smaller when compared to those from the intact ones.

No differences were found between the body weights of intact and adrenalectomized non pregnant rats (Table I). Of the intact animals those in the post partum groups had higher weights than those in the non pregnant one. The adrenalectomized groups showed the same pattern, the weights in the post partum ones being higher.

There were no significant differences in the biomechanical data on the pubic symphysis (Table II) between intact and adrenalectomized non pregnant groups. In the intact group for the third post partum day the elastic stiffness had decreased from the non pregnant state and the first part of the load-deformation curves, the toe part, became lower (Fig 2). However, data from the fifth and tenth post partum days showed a successive restoration. The adrenalectomized rats underwent no such change.

The amount of collagen per unit specimen length in the muscle tendons differed in none of the intact nor adrenalectomized post partum groups from the corresponding non pregnant groups, neither was the amount of collagen in skin changed (Table III).

The biomechanical properties of the muscle tendons in non pregnant rats were not changed by adrenalectomy (Table IV). For the intact rats the elastic stiffness was decreased on the third day of the post partum period and subsequently restored to the level

Table IV Biomechanical properties of peroneus longus muscle tendon (mean  $\pm$  SEM)

Group	$\epsilon_{max}$ (%)	$\sigma_{max}$ (N/mg/mm)	$\tan \alpha$ (N/mg/mm)
Intact			
Non pregnant	0.24 $\pm$ 0.02	158 $\pm$ 23	1.020 $\pm$ 0.68
Post partum			
3 days	0.24 $\pm$ 0.02	123 $\pm$ 12	828 $\pm$ 48
5 days	0.21 $\pm$ 0.02	117 $\pm$ 11	901 $\pm$ 49
10 days	0.25 $\pm$ 0.03	140 $\pm$ 12	955 $\pm$ 119
Adrenalectomized			
Non pregnant	0.24 $\pm$ 0.01	128 $\pm$ 13	851 $\pm$ 97
Post partum			
3 days	0.27 $\pm$ 0.03	131 $\pm$ 19	846 $\pm$ 92
5 days	0.26 $\pm$ 0.03	125 $\pm$ 25	766 $\pm$ 65
10 days	0.23 $\pm$ 0.02	84 $\pm$ 9†	666 $\pm$ 87

‡  $p < 0.05$  against intact non pregnant

†  $p < 0.05$  against adrenalectomized non pregnant

N/mg/mm

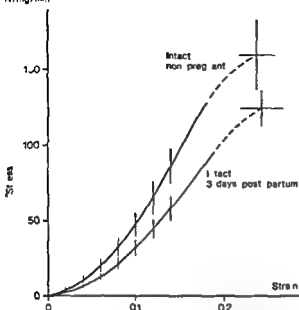


Fig 3 Mean stress strain curves for muscle tendons from non pregnant and 3 days post partum intact animals SEM indicated by bars

found in non pregnant animals. The stress strain curves for the intact non pregnant and third post partum day groups are shown in Fig 3. No decrease occurred in the adrenalectomized animals. Here however the maximum 'stress' was lower on the tenth day than in the corresponding non pregnant group.

N/mg/mm

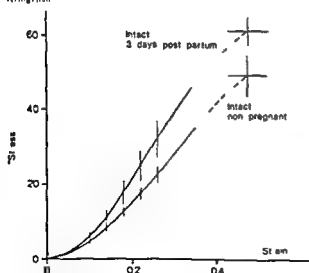


Fig 4 Mean stress strain curves for skin from non pregnant and 3 days post partum intact animals SEM indicated by bars

Table V Biomechanical properties of skin specimens (mean  $\pm$  SEM)

Group	$E_{0max}$ (-)	$E_{max}$ (N $\pm$ SEM)	$E_{10}$ (N $\pm$ SEM)
Intact			
Non pregnant	$0.47 \pm 0.04$	$49.6 \pm 3.3$	$16.1$
Post partum			
3 days	$0.47 \pm 0.04$	$61.4 \pm 3.7$	$24.1$
5 days	$0.46 \pm 0.02$	$54.5 \pm 3.7$	$17.9$
10 days	$0.46 \pm 0.04$	$46.3 \pm 3.3$	$17.2$
Adrenalectomized			
Non pregnant	$0.46 \pm 0.04$	$49.0 \pm 3.3$	$14.1$
Post partum			
3 days	$0.47 \pm 0.05$	$55.3 \pm 6.8$	$20.1$
5 days	$0.47 \pm 0.03$	$54.4 \pm 4.2$	$17.4$
10 days	$0.42 \pm 0.03$	$36.8 \pm 3.3$	$12.4$

2  $p < 0.05$  against intact non pregnant

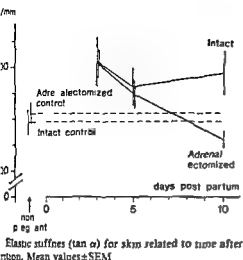
† 2  $p < 0.05$  against adrenalectomized non pregnant

‡ 2  $p < 0.01$  against adrenalectomized non pregnant

For skin there was found with regard to biomechanical parameters no differences between the intact and adrenalectomized non pregnant rats (Table V). For the intact groups the elastic stiffness was found to be increased on the third day after parturition compared with the non pregnant rats. 4) In the adrenalectomized rats the maximum 'stress' and elastic stiffness were reduced on the tenth post partum day compared to the non pregnant rats. These changes in the elastic stiffness during the post partum period are shown in Fig 5. In the intact rats the elastic stiffness was increased on the third day after parturition and remained above the level of the non pregnant state. In adrenalectomized rats the elastic stiffness was increased on the third day after parturition but was subsequently reduced to below the non pregnant state on the tenth day.

## DISCUSSION

Pregnancy influences the functional properties of the collagenous frameworks of the body most notably those in the genital tract but also those of the extragenital tissues (5, 12). In the rat these changes in the pubic symphysis were found by Rundgren (13) to be a decrease of elastic stiffness while for the ligaments there was a decrease of tensile strength. The tensile strength of the dermis was found to be decreased during the first and second post partum period and the post partum period restoration occurred during the



original state in four to five weeks. These findings are confirmed in the present study. For the pubis and muscle tendons of intact animals in the early post partum phase there was a decrease of elastic stiffness followed by a restoration as anticipated. For the adrenalectomized animals, however, there was no such initial decrease, which indicates that relaxation during pregnancy had been less pronounced or absent. The strength of the muscle tendons in these animals, however, was steadily decreased during the post partum period. The skin of the intact animals had increased elastic stiffness in the early post partum period. This reaction was also found in the adrenalectomized animals, but here the strength and stiffness of the skin post partum was not restored to the intact level but became further reduced. On the 10th day after parturition, during the 3–5 days of the post partum period, the concentration of corticosteroids was increased. It might therefore be suggested that the adrenal glands play a role in the stabilization of extragenital collagen in the post partum phase, when large amounts of collagen in the genital tract are catabolized. Corticosteroids and corticotropin increase the mechanical strength of skin (9, 15). Adrenalectomy, on the other hand, has been reported to decrease the strength of skin during the first 15 post operative days (15). However, in this time period the operation itself may influence the connective tissue (10, 14). A vertical incisional wound results in a decrease of the elastic stiffness of skin and also of the denaturing temperature of collagen distant to the wound site.

The thermal contraction force of the rat tail tendons decreases after an incisional wound but returns to normal three weeks later. It is therefore difficult to differentiate between the influences of the operation and the lost adrenal function in the first two weeks after the adrenalectomy. In order to counter any such phenomena, the rats in the present study were therefore adrenalectomized six to seven weeks before the mechanical testing. Here the biomechanical data for the intact and adrenalectomized non pregnant groups did not differ from each other.

It seems that the adrenal glands do not only participate in the restoration of the mechanical properties of the pubic symphysis muscle tendons and skin in the post partum period, but they also play a role in the development of adaptive changes like the relaxation of the pubic symphysis during pregnancy.

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*Submitted for publication January 10 1977*

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## CASE REPORT

MONOZYGOTIC TWINS WITH DISSIMILAR PHENOTYPES  
AND CHROMOSOME COMPLEMENTS

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A case history of monozygotic twins is presented. Twin A showed gonadal dysgenesis and chromosomal mosaicism (45,X0/46,XX). Twin B was a phenotypically normal female with the karyotype 46,XX. Fifteen previous reports of monozygotic twins with gonadal dysgenesis are reviewed. Dissimilar phenotypes seem to be the rule rather than the exception, and mosaicism is found in the majority of cases with dissimilar phenotype. The assumption is made that the chromosome disorder leading to gonadal dysgenesis arises postzygotically either before or after the process of twinning.

monozygotic twins dissimilar phenotypes are exceptional. Twelve cases are listed by Nielsen (8). In five cases one twin had Down's syndrome while the other was normal. In the remaining seven cases one twin had Turner's syndrome while the other was normal. As the incidence of Turner's syndrome is less than one out of 2 500 newborns and that of monozygotic twins with female phenotype one out of 10-800 newborns, the combination of these two conditions should be anticipated in one out of 15-2 million newborns (12). It is our aim to report a new case and to review the previous 15 reports of monozygotic twins with gonadal dysgenesis.

## CASE REPORT

A 16-year-old patient was referred to our department for further evaluation of primary amenorrhea. Both parents and a sister two years older were healthy. The mother had at age 35 years old when she became pregnant again. The pregnancy was uneventful, and the delivery took place at home. We have little information about the delivery but it is known that the patient was born as twin A. Twin A was always smaller than her twin sister but otherwise she developed normally until puberty. When admitted her height was 137 cm and her weight 38.8 kg. The neck was short, but there was no webbing. The breasts were not

developed, the external genitalia were infantile and no axillary or pubic hair was seen. In contrast her twin sister was 162 cm high, had a normal female appearance and had menstruated regularly since the age of 11 years. Fig. 1 shows the twin sisters when they were 18 years old.

Hormonal investigations in twin A showed that the urinary excretion of total estrogens was  $< 2 \mu\text{g}/24 \text{ h}$  determined by the method of Brown *et al.* (1) while the urinary excretion of total gonadotropins was 76-140 mouse uterine units/24 h determined by the method of Johnsen (4). Both thyroid and adrenal function was normal.

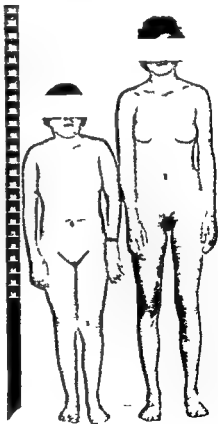


Fig. 1 Twins A and B when 18 years old

Table I Cytogenetic studies of twins A and B

Subject	Buccal smear		Lymphocytes in peripheral blood					Skin					
	Number of chromatin pos	Number of cells counted	43	44	45	46	47	Number of cells examined	43	44	45	46	47
Twin A	a	5				9	21	30			5	15	30
	b	2				9	20	1					
Twin B	17	100	1	1	1	1	1	20			not examined		

X rays of the right hand showed that the bone age was retarded by 3 years while X rays of the skull chest and kidneys were all normal

An exploratory laparotomy revealed a hypoplastic uterus and typical streak gonads. The streaks were removed and histological examination showed fibrotic tissue without any follicles

**Cytogenetic studies** The results are shown in Table I. In twin A both a buccal smear and lymphocytes from peripheral blood were examined twice (a and b). The cells with the modal number 46 had a normal female sex chromosome complement whereas the cells with the modal number 45 had an XO complement. Analysis of cells with modal numbers differing from these suggested that they were artefacts during preparation. It is concluded that twin A is a 45 XO/46 XX mosaic and twin B a chromosomally normal female.

**Zygoty studies** Determination of erythrocyte phenotypes and tissue types of both twins and their parents was performed. The twins had identical blood groups (Table II).

The probability of monozygosity based on the blood groups is 99.37 per cent according to the method described by Smith & Penrose (15). Furthermore a full thickness skin graft experiment taking skin from twin A and grafting it onto twin B and vice versa was carried out. Both grafts

and a complete take and no rejection occurred during an observation period of 8 weeks. This indicates a permanent survival as could be anticipated in monozygotic twins.

## DISCUSSION

Until now 16 cases have been reported of monozygotic twins in whom one or both twins had gonadal dysgenesis and a 45 XO karyotype at least in some of the tissues. Reviewing these cases (Table III) we found 4 cases of identical phenotype and gonadal dysgenesis in both twins. Including our own case 12 cases had identical phenotypes. In 11 of these cases one twin had gonadal dysgenesis while the other twin was a phenotypical normal female and in 4 cases one twin was a phenotypical normal male. In one case reported by Russell *et al* (12) one twin had gonadal dysgenesis while the other twin showed male intersexuality. In one case one gonad was an immature testis and the other a streak ovary. Edwards *et al* (3) report a case where one of the monozygotic twins had gonadal dysgenesis and the karyotype 46 XY/45 XO in blood and skin. The other twin was a phenotypical normal male with the karyotype 45 XO in both blood and skin. No 46 XY cells could be demonstrated in the skin. The investigators draw the conclusion that the cells available for culture were not necessarily representative of those determining the difference in phenotype.

Table II Erythrocyte phenotypes and tissue types of twins A and B and their parents

Subject	Blood types										Tissue types		
	ABO	Rh	MNS	P <sub>1</sub>	Lu <sup>a</sup>	Lu <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	Fy <sup>a</sup>	Fy <sup>b</sup>		Xg <sup>a</sup>	
Twin A	A <sub>1</sub>	R <sub>1</sub> R <sub>1</sub>	MNS	+	-	-	+	-	-	+	+	A <sub>1</sub> MNS++	C+CW-D+E-c-P+X Hpa 21 Gc 1 Gcs 1 PGM 11 SP B AK 11 PGD A ADA 11
Twin B	A <sub>1</sub>	R <sub>1</sub> R <sub>1</sub>	MNS	+	-	-	+	-	-	+	+	A <sub>1</sub> MNS++	C+CW-D+E-c-P+X Hpa 21 Gc 1 Gcs 1 PGM 11 SP B AK 11 PGD A ADA 11
Mother	A <sub>1</sub> B	R <sub>1</sub> r	MNS	+	-	-	-	+	+	+	+	A <sub>1</sub> BMS++	C+CW-D+E-c-P+X Hpa 21 Gc 1 Gcs 1 PGM 11 SP B AK 11 PGD AB ADA 11
Father	A <sub>1</sub>	R <sub>1</sub> r	MNS	+	-	+	+	+	-	+	-	A <sub>1</sub> MNS++	C+CW-D+E-c-P+X Hpa 21 Gc 1 Gcs 1 PGM 11 SP B AK 11 PGD A ADA 11

Table III Review of 16 cases of monozygotic twins with gonadal dysgenesis

Reference	Karyotype		Gonads	Other	Phenotype
	Blood	Skin			
Turner & Zanartu	45 XO	45 XO			Turner's syndrome
6	45 XO	45 XO			Turner's syndrome
Smith & Smith	45 XO				Turner's syndrome
63	45 XO				Turner's syndrome
Decourt <i>et al</i>	45 XO				Turner's syndrome
64	45 XO				Turner's syndrome
Ekhof <i>et al</i>	45 XO	45 XO			Turner's syndrome
72	45 XO	45 XO			Turner's syndrome
Wright <i>et al</i>				45 XO	Turner's syndrome
61				46 XY	normal male
Wikelsen <i>et al</i>	46 XX/45 XO	46 XX/45 XO			normal female
81	46 XX/45 XO	46 XX/45 XO			Turner's syndrome
Edwards <i>et al</i>	46 XY/45 XO	45 XO			Turner's syndrome
66	45 XO	45 XO			normal male
	46 XX/45 XO				normal female
	46 XX/45 XO				Turner's syndrome
Wells <i>et al</i>	45 XO	45 XO			Turner's syndrome
83	45 XO	46 XY/45 XO	46 XY/45 XO		Turner's syndrome
Wine & Cornery	46 XX				male intersexuality
66	-				normal female (Xga pos)
					female with multiple abnormalities (Xga neg)
Wass <i>et al</i>	47 XXX/45 XO	47 XXX/45 XO			Turner's syndrome
69	47 XXX/45 XO	47 XXX/45 XO			normal female
Wier & Taitz	46 XX/45 XO	45 XO			Turner's syndrome
71	46 XX/45 XO	46 XX			normal female
Wrubina <i>et al</i>	46 XX/45 XO				normal female
75	46 XX/45 XO				Turner's syndrome
Wright <i>et al</i>	46 XY				normal male
75	46 XY		45 XO	46 XY/45 XO	Turner's syndrome
Wright <i>et al</i>	46 XY	45 XO	45 XO		Turner's syndrome
76	46 XY				normal male
Recent report	46 XX/45 XO	45 XO			Turner's syndrome
	46 XX				normal female

In the four cases with identical phenotype no mosaicism was found. In 10 of the twelve cases with dissimilar phenotype mosaicism was found in one or both twins. Since mosaicism was present in most monozygotic twins with gonadal dysgenesis, both twinning and chromosomal disorder must be postzygotic events.

In the present case mosaicism was only found in one twin with gonadal dysgenesis. If this is true it seems that twinning took place before the chromosomal disorder leading to the gonadal dysgenesis. Another possible explanation is that both twins are mosaics but that the mosaicism in twin B remained undetected because only the blood was examined.

#### ACKNOWLEDGEMENTS

The authors wish to thank A. Svejgaard MD (Laboratory of Blood and Tissue Types, Rigshospitalet, Copenhagen) for the determinations of erythrocyte phenotypes and tissue

types and M. Pers MD (Department of Plastic Surgery, Rigshospitalet, Copenhagen) for carrying out the skin graft experiments. The statistical analysis was performed by B. Bjerregaard MD (Department of Obstetrics and Gynecology, Rigshospitalet, Copenhagen).

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*Submitted for publication December 10 1975*

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## CASE REPORT

## ANTENATAL ULTRASONIC DIAGNOSIS OF COMPLETE URETHRAL OBSTRUCTION IN THE FETUS

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A case of ante natal diagnosis of complete obstruction of the urethra is reported. By repeated ultrasonic B-scans a distended bladder filling the abdominal cavity could be seen and a hydroureter was demonstrated. Early diagnosis of this condition which is incompatible with life, a decision to terminate pregnancy can be made.

fetal urinary bladder should be given special attention during routine ultrasound scanning as its size of filling can help the examiner to detect different fetal malformations (1-3). Complete obstruction of the urethra is a very rare condition and it causes severe distention of the urinary bladder, bilateral hydroureter and hydronephrosis with constant oligo-amnios.

Three cases of such a malformation detected by ultrasonographic B-scan have appeared in the literature: two of them were diagnosed antenatally and in the third case the final diagnosis was established only at delivery. Garret *et al* (2) described two cases: one with a complete obstruction and a stillborn infant while the other had only a partial stricture followed by a live birth. Okulski (6) reports on a urethral obstruction in the fetus with a prune belly syndrome but the degree of obstruction is not clear. The infant died at the age of two months. In Nevins *et al* (5) an absence of the urethra was found with an extremely distended bladder and other malformations in different organs. The infant died at birth. A case of complete obstruction of the urethra is reported here stressing the importance of the possibility of early detection of such malformations.

## CASE REPORT

A Jewish Yemenite woman aged 25 was referred to the high risk pregnancy clinic when 22 weeks pregnant (according to the LMP) as the clinical impression was that the fetus was larger than expected for the gestational age. In

the past she had had a spontaneous abortion and a normal delivery of a healthy infant. All general laboratory and physical examinations were normal. A grey scale ultrasound B-scan revealed a biparietal diameter (BPD) of 54 mm corresponding to 21 weeks of pregnancy, no amniotic fluid and a highly distended bladder filling almost the whole fetal abdominal cavity. The intestines were packed tightly towards the spine and the diaphragm and some fluid was seen in the pleural cavity (Fig 1). An attempted amniocentesis failed as no amniotic fluid could be withdrawn. Repeated scans showed the same huge urinary bladder (Fig 2). At 28 weeks amenorrhea and an additional distended body was seen which was assumed to be a hydroureter (Fig 3). With the progress of the pregnancy the picture of intrauterine growth retardation became more evident. At 35 weeks the BPD corresponded to 30 weeks. Urinary estriol and non stress cardiotocography revealed nothing abnormal but fetal movements as reported by the patient decreased. At 36 weeks after spontaneous labor the patient delivered a male fetus weighing 2 200 g through a partial breech extraction which died a few minutes after birth. Physical examination revealed a much enlarged abdomen, severe dehydration and a face which was reminiscent of the Potter syndrome. Post mortem examination (Fig 4) showed

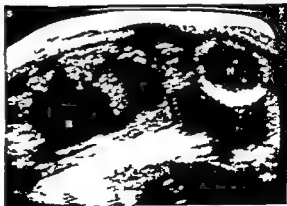


Fig 1 Longitudinal ultrasonic scan at 22 weeks with distention of the fetal bladder and oligo-amnios. H = head, B = bladder, X = xyphoid area of mother, S = symphysis pubis of mother.



Fig 3 Transverse ultrasonic scan of the fetal upper abdomen with a huge bladder and probable hydroureter B=bladder K=ureter I=intestines L=left R=right



Fig 4 Anatomical preparation of the fetal trunk with a large bladder and bilateral hydronephrosis B=bladder U=ureter K=kidney

a distended urinary bladder, total atresia of the membranous part of the urethra, partial atresia of the part of the left ureter due to mural valve lesion, and partial stenosis of the lower part of the right ureter at the uretero-vesical junction. There was bilateral hydronephrosis with atrophy of the renal parenchyma.

## DISCUSSION

In the Potter syndrome with bilateral agenesis of the kidneys, the ultrasonic ante-natal diagnosis is based on unrecognizable fetal kidneys and a bladder with oligo-amnios and failure to demonstrate a distended bladder after administration of furosemide to the mother (4). There is also intra-uterine growth retardation. In the syndrome of complete obstruction of the urethra, the diagnosis is made by observation of a

re distension of the bladder which may fill the whole abdominal cavity and sometimes hydroneph

5 This malformation causes severe secondary changes in the urinary tract incompatible with postnatal life and interruption of pregnancy should be considered when the diagnosis is confirmed by ultrasound. In this case the pregnancy was allowed to continue owing to the lack of previous knowledge. The obstetricians were not absolutely certain of the diagnosis. With the description given in this report it is to be hoped that more accurate diagnosis will allow an early decision to terminate such hopeless pregnancies.

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*Submitted for publication September 6, 1979*

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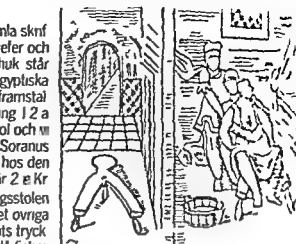
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## CASE REPORT

## PAPILLARY CYSTADENOFIBROMA OF THE ENDOMETRIUM

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Six cases of papillary cystadenofibroma of the endometrium were found in the medical literature. A seventh of this kind of tumor is now described clinically, histologically and the literature pertinent to this particular tumor reviewed.

**eponym** Papillary Adenofibroma (1) characterizes a tumor having a fibroepithelial nature and resembles a tumor having a fibroepithelial nature. At the first cases of papillary adenofibroma of cervical origin were reported in 1971 by Abell. Eleven reported cases of such tumors in the uterus (1, 3, 7, 8). 5 of them arising from the cervix and 6 from the endometrium (3, 7, 8) are to be found in the medical literature. Because of the characteristic feature of this kind of tumor having only papillary structures protruding into cystic spaces. Grimalt (3) recommended the term papillary adenofibroma.

A seventh case of papillary cystadenofibroma of the endometrium, the largest in this site hitherto and was considered worthwhile reporting.

## CASE REPORT

A 71 year-old nulliparous widow complained of a prolonged vaginal discharge. Pelvic examination revealed an enlarged uterus, the size of a 12 week pregnancy. The cervix was effaced and dilated to 1 cm by the lower pole of a lobulated, granular, elastic tumor. A total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed after biopsy of the mass had revealed a malignant tumor. Recovery was uneventful and the patient was discharged on the 7th postoperative day. Half a year after the operation the patient feels well and has no complaints.

**Microscopic findings** An enlarged uterus (size 16x10x7 cm) together with adnexae were received for examination.

The uterine cavity was entirely filled by a tumor which protruded into the external cervical os (Fig. 1). The tumor itself was polypoid measuring 14x8x5 cm and was attached by a broad base to the uterine fundus. Its surface was irregular having many round and ovoid nodules. On its cut surface the tumor had a spongy consistency and contained mucoid fluid within multiple cystic cavities measuring 0.1-1.5 cm in diameter (Fig. 2). The uterine wall was 1.5 cm thick with atrophic endometrium covering the tumor free parts. On its left lateral wall a spongy sessile polyp was found 3 cm in diameter not contiguous with the larger polypoid tumor. The Fallopian tubes and ovaries were quite normal.

**Microscopic findings** Both fibrotic stroma and epithelium were present in the polypoid tumor. Cysts and glands of varying size and shape were seen embedded in a fibroblastic stroma throughout the entire specimen. Blunt papillary folds protruding into cystic spaces were a general characteristic feature of the tumor (Fig. 3). Tall columnar and cuboidal epithelium resembling endocervix lined most of the cystic cavities, glands and papillary projections (Fig. 4 and inset). This epithelium was flattened in the larger cysts. Large amounts of mucicarmophil material was present in the cytoplasm of the lining epithelium and in luminae of the

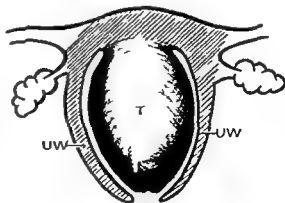


Fig. 1 Schematic sketch of specimen before incision. UW uterine wall, T tumor.



Fig 2 Gross appearance of the resected specimen. Left side: a polypoid mass attached by a broad base to the fundus of the uterus. Right side: cross section shows many cystic spaces, variable in size, giving a spongy appearance to the tumor.

glandular and cystic spaces. A few glands and cysts lined by endometrial epithelium were observed in the area where myo- and endometrium were contiguous with the polypoid neoplasm. The outer surface of the tumor was covered with the same endocervical epithelium. In most areas the fibrous stroma adjacent to the epithelium of these structures was composed of small, loosely arranged fibroblasts, while more compound spindle and stellate fibroblasts made up the deeper cellular region (Fig 3). The nuclei of the stromal cells were uniform and small, demonstrating in some places storiform whorled patterns. In other areas the cells were hyperchromatic, with vesicular nuclei of irregular size and shape, having single or multiple nucleoli, characteristic of nuclear atypia (Fig 5 and inset). The small separate sessile polyp on the uterine wall had a similar microscopic appearance. The endometrium demonstrated senile cystic atrophy.



Fig 3 Fibrocellular papillary folds projecting into cystic space. Note the abundant mucous secretion. Stroma adjacent to the epithelium is composed of loosely arranged small fibroblasts, continuing closely arranged, forming a dense stroma. Hematoxylin and eosin  $\times 80$ .

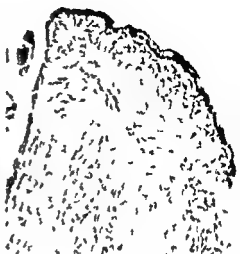


Fig 4 Papillary fold composed of loosely arranged fibroblasts covered by columnar endocervical epithelium. Hematoxylin and eosin  $\times 230$ . (Inset) Higher magnification of the columnar secreting surface epithelium. Hematoxylin and eosin  $\times 360$ .



Fig 5 Areas in which the stroma shows nuclear atypia. Fibroblasts are packed closely together. Hematoxylin and eosin  $\times 200$ . (Inset) Higher magnification of the hyperchromatic nuclei of the stromal cells. Hematoxylin and eosin  $\times 360$ .

## DISCUSSION

(1) described three polypoid lesions of endocervical origin which differed microscopically from the endocervical mucosal polyp and its variants. He referred them to be true neoplasms and termed papillary adenofibroma. These tumors bore a resemblance to the adenofibroma of ovary, intestine and breast. Vellos *et al* (8) described 4 of them arising from the endometrium. In their opinion this tumor is a benign counterpart of the malignant mixed mesodermal tumor of the uterus of Müllerian origin. Malt *et al* (3) described the electron microscopical as well as the histochemical characteristics of a papillary adenofibroma of the endometrium. The tumor consisted exclusively of fibroblasts associated with active fibrillogenesis. The covering epithelium of the papillary cystadenofibromas of the mucinous secretory type and showed substructural similarities to the normal endometrial (2) and Bartholin gland epithelium (5). Further confirmation of its close resemblance to endocervical epithelium was provided by the histochemical studies which revealed the presence of abundant sialic and carboxylic mucins in the epithelium. Secretions of similar findings were reported in the normal cervical epithelium (6). Endometrial stromal (1) and foci of squamous epithelium (8) were found also as a covering epithelium of some papillary cystadenofibromas. The mucous covering epithelium is the result of metaplasia of the cervical epithelium. Similar metaplastic processes occur in other endometrial lesions including mucous polyps and primary endometrial carcinoma (6). The multipotential nature of the endometrium during the Müllerian duct system explains the metaplastic transformation of endometrial stroma into endocervical tubal or squamous epithelium. In our case all the lining epithelium proved to be of the mucus secreting type. A direct histological continuity between the polypoid neoplasm and the underlying endo- and myometrium was noted and in these areas a few glands and cysts lined by endometrial epithelium. This supports the theory that the mucus secreting epithelium of the endometrial glands is the result of metaplasia of the predominant fibroblastic growth and intricate structural organization of papillary cystadenofibromas suggests a neoplastic rather than a hyperplastic epithelial endometrial stromal cell proliferation. Under these circumstances the covering epithelium ap-

pears to be a passive component. If this is the real explanation then cystadenofibroma of the uterus is not the benign counterpart of malignant mesodermal tumor (8) where both components (mesenchymal and epithelial) are regarded as actively growing neoplastic elements.

The detection and recognition of papillary cystadenofibroma of the endometrium is important mainly for the interpretation of material obtained by uterine curettage biopsies. Confusion may arise between this lesion and that of endometrial stromatosis. Both occur in pre- and postmenopausal women presenting with uterine bleeding. In several cases endometrial stromatosis was described macroscopically as a polypoid mass protruding into and filling the endometrial cavity. Microscopically the tumor consists of well differentiated proliferating fibroblasts. However endometrial stromatosis is devoid of epithelial elements which seem to be the only reliable criterion for differentiation between these two lesions. Nevertheless there are cases where endometrial glands may be surrounded and compressed by neoplastic stromal cells (4). The absence of mitoses is not a reliable criterion since endometrial stromatosis may also lack mitotic figures and the differential diagnosis then becomes more difficult when the fibroblastic growth in papillary cystadenofibroma displays nuclear atypia as illustrated in our case.

All the reported cases of uterine papillary cystadenofibroma were of benign character both histologically and clinically. Consequently the recognition and diagnosis of such lesions can be crucial in deciding the proper way to treat such patients and in predicting their prognosis.

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*Submitted for publication June 2 1979*

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## CASE REPORT

## HORMONE TREATMENT OF STROMAL ENDOMETRIOSIS

Jens Brøns Lene Kjaer Jensen and Jan Rasmussen

*From Radiumstationen and the Department of Pathology FinsenInstitutet Copenhagen Denmark*

**Abstract** A report of two cases of recurrent stromal endometriosis both the patients having positive estrogen receptors in the tumor tissue. They were first treated with mifepristone which failed to halt growth of the tumor. After a second subsequent treatment with progesterone the tumor regressed and the patients' condition improved.

Stromal endometrial tumors are rare and constitute only 0.2 per cent of all uterine malignancies (3-4). Morpho-anatomically the tumors develop from the stromal cells of the endometrium without associated endometrial glands. The clinical symptoms of these tumors are somewhat variable but are generally uncharacteristic, the most frequent symptom being menorrhagia. An ambiguous nomenclature has led to much confusion. Among synonyms and related terms to be mentioned are stromatosis, endolymphatic stromal myositis, stromatous endometriosis, and endometrial interstitial (7). Stromal endometriosis is often diagnosed as a low grade sarcoma with a smooth transition to real sarcomas. The literature concerning stromal endometriosis is scanty and we have found only very few descriptions of the effect of progesterone on the tumor. Two cases of stromal endometriosis treated by progesterone are presented and discussed.

**Case 1** A 27 year old patient was admitted to the gynecological department of the local hospital. Previously she had been essentially healthy. Menarche had occurred at the age of 13 and after that menstruation was normal. On June 26 she had a normal partus (1963). In November 1964 she was subjected to curettage because of menorrhagia of one month's duration. Histology showed a hyperplastic corpus endometrium. As the menorrhagia continued and it was thought to be caused by fibromyomas, a total hysterectomy was carried out. Histology showed stromal endometriosis with a homogeneous benign impression, but with local invasive growth into the serosa in a few

places. It was limited to the corpus of the uterus and during the operation no signs of spread to the abdomen were found. The patient remained well until November 1975 when she developed a colovaginal fistula. A resection of the colon sigmoideum and closing of the fistula was carried out. A radical operation was not possible owing to the presence of tumor masses in the small pelvis. Histology showed stromal endometriosis with dedifferentiation more pronounced relative to that in earlier biopsies, and tumor in the colon and vagina. No postoperative problems arose and the fistula remained closed. From April 6 1975 to January 13 1977 the patient was treated with estradiol tablets 2 mg (Progynon<sup>®</sup>) 1 tablet daily because of climacteric symptoms. On examination in January 1977 a relapse was observed and the patient was referred to the Radiumstationen FinsenInstitutet Copenhagen. On January 14 1977 a polypoid tumor measuring 3 x 2 cm was found in the top of the vagina which was fastened to the left side of the pelvic wall by a tumor mass the size of a mandarin orange. Histology showed stromal endometriosis. In addition estrogen receptor concentration was estimated. It was  $4.7 \times 10^{-14}$  mol/mg protein i.e. positive (lower limit  $7 \times 10^{-14}$  mol/mg protein). Progesterone receptor was not estimated. An i.v. pyelogram which earlier had been normal now showed hydronephrosis on the left side. From January 14 to February 8 1977 the patient was treated with tablets hydrocortisone 20 mg (Hydrocortison<sup>®</sup>) 1 tablet x 3. During this period gynecological examinations showed no change. Because of the positive estrogen receptor result the patient was treated with tablets tamoxifen 10 mg (Nolvadet<sup>®</sup>) 1 tablet x 3 from February 8 to March 8 1977. In this period the patient's condition deteriorated with vaginal bleeding, abdominal pains and progression of the tumor. Since the tumor now measured 4 x 3 cm and the infiltration in the small pelvis had spread to the posterior wall, treatment with tablets medroxyprogesterone acetate 100 mg (Climovir<sup>®</sup>) 1 tablet daily was started on March 22 1977 and still continues. The patient's condition improved drastically after this change of treatment and the abdominal pain and vaginal bleeding ceased within 14 days. The patient has been examined regularly since and the size of the tumor has diminished steadily. By October 26 1977 there was no sign of tumor in the vagina and no tumor masses were palpable in the small pelvis. An i.v. pyelogram on December 18 1977 was normal. Roentgen examinations of the chest have always been normal. At the last gynecological examination there was no sign of tumor and the patient felt well.



Fig 1 Patient 1 Compact masses of spindle shaped cells permeate the myometrium along or within the lymphatics veins or tissue spaces and herniate into the vessels (Original magnification  $\times 25$ )

**Patient 2** The patient 46 years old, was admitted to the gynecological department of the local hospital. Formerly she had been essentially healthy. The menarche had occurred when the patient was 14, since then menstruation had been regular. The patient had never been pregnant. In 1970 she had metrorrhagia and due to suspicion of fibromyomas a total hysterectomy was carried out. Histology showed stromal endometriosis without signs of invasive growth. There were, however, islands of stromal tissue in relation to veins and lymphatics. At operation there was no sign of spread to the abdomen. Because of climacteric symptoms the patient was treated with injections of estradiol benzoate 10 mg (Follicylin<sup>(R)</sup>) every 2–3 months from 1974 to April 1976. In March 1976 the patient had abdominal pain and yellow brown fluor vaginalis and on gynecologic examination a  $1 \times 3$  cm polypoid tumor was found in the top of the vagina connected to a mass in the small pelvis. At explorative laparotomy a  $3 \times 3$  cm metastasis on the peritoneum was found besides tumor tissue in the pouch of Douglas and on the intestines. Biopsy showed stromal endometriosis as previously. On July 22 1976 the patient was examined at the Radiumstationen FinsenInstitutet for the first time as she still had abdominal pain. On gynecologic examination a  $3 \times 1\frac{1}{2}$  cm vaginal tumor was found which was fixed to the right wall of the pelvis by a smaller mass. Biopsy showed stromal endometriosis. Estrogen receptor estimation was positive —  $4.2 \times 10^{-14}$  mol/mg protein. Progesterone receptor estimation was negative. The first treatment was i.v. injections of Rubidaxon 200 mg from July 28 to December 1 1976. During this period the tumor remained stationary but the patient still had pain. The treatment was stopped because of ECG changes. Due to the positive estrogen receptor result treatment with tablets tamoxifen 10 mg (Nolvader<sup>(R)</sup>) 1 tablet  $\times 3$  was given from February 1 to March 2 1977. This resulted in significant deterioration of the patient's symptoms with vaginal bleeding increased abdominal pain and progression of the tumor which now measured  $3 \times 3 \times 11$  cm and was fixed to the pelvic wall on both sides. On March 24 1977 treatment with tablets medroxyprogesterone acetate 100 mg (Clinoval<sup>(R)</sup>) 1 tablet

daily was started. Within the first month the condition improved significantly the vaginal bleeding and abdominal pain ceased. The patient was content with the size of the tumor was observed to decrease. At the last control on July 11 1978 the tumor measured  $\times 2$  cm and nothing abnormal was found in the pelvis. The patient remains in good condition and symptoms continues. Roentgen examination of the chest at all the time been normal. i.v. progesterone has been discontinued.

## DISCUSSION

The long symptom free periods in the two cases respectively 11 and 6 years before relapse is characteristic. This is in accordance with the report (1) in which relapse occurred up to 20 years after the diagnosis of stromal endometriosis was made. Both our patients were under estrogen treatment for 1 and 2 years respectively before relapse appeared. The role this treatment plays in delaying or accelerating the growth of recurrence is uncertain but an earlier report has shown that the growth of this kind of tumor may be stimulated by estrogen. Successful treatment of stromal endometriosis with progesterone was first described in 1968 by Pöhlert when a patient with lung metastases remained symptom free after 7 years of treatment and was considered completely cured. In the literature we have been able to find 3 additional cases of stromal endometriosis treated with progesterone. A good response was mentioned in all the cases when complete disappearance of the tumor in our patients the tumor disappeared completely in one case and diminished drastically in the other which supports the general impression that progesterone is

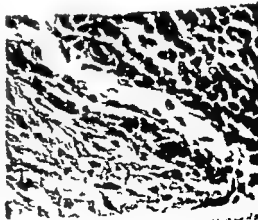


Fig 2 Patient 2 Spindle-shaped cells permeate the endometrial stroma and have a large vessel or space in the lower left field (Original magnification  $\times 25$ )

ory effect on endometrial stromal tumors. Estimations on this type of tumor not been performed before and the positive suggest that the antiestrogen therapy with was effective. On the other hand the treatment with antiestrogen had failed to the growth of the tumor or the deterioration of its symptoms.

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*Submitted for publication January 16 1979*

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1982

Date	Place	Name	Office
<b>1980</b>			
October 3-5	New Delhi India	3rd International Seminar on Maternal and Perinatal Mortality Pregnancy Termination and Sterilization	Hon. General Secretary The Fed of Obstetric & Gynecological Societies of India Purandare Group III Dr N A Purandare M.A. Bombay 400 007 India
Oct-Nov 23-3	San Marino Italy	The Gonadotropins Basic Science and Clinical Aspects in Females	Leslie Nies Symposia Manager Sem Symposia 11 Brooks Drive, Braintree MA 02184 USA
November 14-17	Madrid Spain	7 Congrès Européen de Médecine Périnatale	Professor de la Fuente Maternidad de la Ciudad Av Generalísimo 177 Madrid 28.014
November 18-23	New Orleans LA USA	AAGL Ninth Annual Meeting Clinical Symposium on gynecologic endoscopy	American Association of Gynecology Laparoscopists 11439 South Latwood Boulevard Downey CA 90244 USA
November 20-22	Barcelona Spain	Symposium Internacional Sobre Monitorización Prenatal	Instituto Dexeus Srtas M Lluís Lluís Ana Baldrich c/Paseo de la Bonanova 67 Barcelona 17 Spain
November 22-25	Bombay India	First National Congress on Hormones and Human Reproduction	Mahendra N Panikkar Organizing Secretary Dr Jhaveri Hospital 24 Lady Hardinge Road Bombay 400 001 India
December 1-4	Kairo Egypt	Second Congress of the International Society for the Study of Hypertension in Pregnancy	Docent Hjördis Robbe Dept of Obstet and Gynecol Karolinska Hospital S-104 01 Stockholm 80 Sweden
December 1-12	Melbourne Australia	Seventh UICC Training Course in Cancer Research	Dr A W Burgess UICC Course The Walter and Eliza Hall Institute Royal Melbourne Hosp PO Box 1207 Victoria Australia

## CASE REPORT

## ENDOMETRIOID CARCINOMA IN AN OPERATION SCAR

Hans Madsen Peer Hansen and Ole Peter Andersen

*From the Department of Obstetrics and Gynecology Aalborg Hospital Denmark*

**Abstract** A case of primary endometrioid carcinoma in endometriosis in an operation scar is described

Endometriosis is a common disorder most frequently found in the ovaries uterosacral ligaments and peritoneum Endometriosis is seldom found in an operation scar (3) Development of carcinoma in endometriosis is extremely rare Endometrioid carcinoma most commonly occurs in the ovary (2) extraovarian lesions have only been observed in very few cases and then only in the rectovaginal septum (2) however a few cases of endometrioid carcinoma in the pleura have been described (1) Endometrioid carcinoma developing within endometriosis in an operation scar has not previously been reported

## CASE REPORT

A 39-year-old woman was admitted with a history of abdominal tumor She also complained of increasing abdominal pain extending over six months and a swollen abdomen She had had six normal deliveries and 17 years previously had an abortion by means of a hysterotomy combined with sterilization No gynecological symptoms were noted except for menopausal hot flushes during the preceding year Her menstrual cycle was regular apart from months amenorrhea six months ago and she was receiving no hormonal therapy Since her operation 17 years ago slight pain in the scar at the time of menstruation had been noted

Abdominal examination revealed a tumor of 12x12 cm in the operation scar Pelvic examination was normal A biopsy from the tumor revealed carcinoma on histological examination presumably a metastasis from ovarian carcinoma Tumor located in the left rectus abdominis was removed from the abdominal wall The tumor contained a dark brown fluid Apparently the tumor was radically removed Both ovaries appeared to be normal with no sign of endometriosis Light microscopic examination concluded mucinous uterine carcinoma developing in endometriosis in the wall as the connective tissue contained

cysts with an endometrioid appearance and exhibited an interval phase which corresponded with the patient's menstrual cycle The same histological characteristics were observed by electron microscopic examination

Röntgen ray examination of the colon was normal Fractional uterine curettage disclosed by histological examination endometrium in the secretory phase without premalignant alterations

Postoperatively the patient was treated by radiotherapy in a dose of 5 500 rads in the abdominal field and 1 600 rads in the pelvic field Castration was additionally performed The patient was in perfect health at the conclusion of treatment

## DISCUSSION

Based on clinical tests operative findings and histology this case undoubtedly represents a primary endometrioid carcinoma developing in endometriosis of an operation scar

There are two main theories about the genesis of endometriosis

1 The metastasis theory proposes that the endometriotic tissue in some way is transported to an extrauterine location

2 The metaplasia theory suggests that the endometriotic tissue arises by metaplasia of the cells in some extrauterine location

Steck & Helwig (3) describe a combination of these two theories in that endometrial cells themselves stimulate imitative metaplasia when they are transported by any means to a susceptible tissue

Due to the implantation of endometrial tissue in the scar at the time of operation the origin of the endometriosis in the present case appears to be in accordance with the metastatic theory

The reason for the development of carcinoma in endometriosis is unknown but it seems quite probable that important factors concerned with the development of carcinoma in the endometrium of the corpus uteri also are important for development of endometrioid carcinoma

# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1980 - 1982

Date	Place	Name	Office
<b>1981</b>			
January 26-31	Mexico City Mexico	Pan American Congress of Andrology	Gerald Bagatznik Cong. Andrology 31600 West Chicago, Illinois, U.S.A.
March 14-18	Atlanta USA	37th Annual Meeting of the American Fertility Society	The American Fertility Society 1608 13th Avenue South, Suite 111 Birmingham Alabama 35204 USA
March 22-26	Berlin West-Germany	IIIrd World Congress of Human Reproduction	Dorent L. Mettler Fraunhofer, de L Hegenscher 4 D-7300 Kiel
March 27-28	Modena Italy	Symposium on Recent Advances on Pathophysiology of Amniotic Fluid	Scientific Secretariat Dr. U. C. Di Ferranti Istituto di Clinica Ostetrica e Ginecologica - Policlinico Via del Pizzardi 41100 Modena Italy
April 15-17	Gorizia Italy	International Course on Ultrasound in Obstetrics	Filippo Destro Organizing Secretary Dept. of Obstetrics and Gynecology City Hospital Via Vittorio Veneto 17 34170 Gorizia Italy
May 17-24	Dubrovnik Yugoslavia	4th European Congress on Ultrasonics in Medicine	Professor Asim Kurjak Lj. Huk Stube 41000 Zagreb Yugoslavia
June 9-12	Ostend Belgium	Third International Congress on the Menopause	The International Menopause Society 8 av. Don Bosco 1140 Brussels, Belgium
July 1-4	Graz Austria	First International Symposium on Minimal Invasive Cancer (Microcarcinoma)	Secretariat First Symposium on Minimal Invasive Cancer P.O. Box 1014 A-1014 Wien Austria
August 24-28	Cambridge England	XIII Acta Endocrinologica Congress	Conference Services Ltd XIII Acta Endocrinologica Congress 3 Bels Lane London SW7 3ET England
Sept-Oct	Athens Greece	Vth European Congress on Sterility (ESCO)	Secretariat Prof. Dr. K. Senn, Frauenklinik der Universitat Kiel, Hopfenstrasse 4 2300 Kiel 1 West Germany
October 25-31	Melbourne Australia	Eight Asian Congress of Obstetrics and Gynecology	The Organizing Secretary VI Asian Congress of Obstetrics and Gynecology G.P.O. Box 195T Melbourne 3001 Victoria Australia
<b>1982</b>			
October 17-22	San Francisco CA USA	Xth World Congress of Gynecology and Obstetrics	Xth World Congress of Gynecology and Obstetrics c/o The American Congress of Obstetrics and Gynecology One East Wacker Drive Suite 202 Chicago Illinois 60601 USA

## ANTENATAL CARDIOTOCOGRAPHY AND INTRAUTERINE DEATH

Thore Söhm and Nils Otto Sjöberg

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The purpose of the present study was to elucidate the reliability of antenatal cardiotocography (CTG) in predicting fetal death. During a 4-year period 1455 patients with risk pregnancies have been routinely monitored with CTG. The total number of tracings amounted to more than 10000. Five cases of fetal death occurred in all with pathological CTG patterns were found. Our experience in the present study indicates that antenatal CTG is a reliable technique for the predicting of fetal death and its use should reduce fetal mortality rates.

Cardiotocography (CTG) was introduced in the sixties after fundamental studies by Caldeyro Barcia, Hammacher and Hon (2, 10, 12). Although interest focused mainly on intrapartum CTG monitoring antenatal monitoring soon attracted attention too (13). The interpretation and value of antenatal CTG has been a matter of dispute and Beard (1) reported in 1974 that experience of antenatal CTG was too limited to allow of an evaluation of its reliability. During recent years more experience has accumulated and reports have been published on the subject of interpretation of CTG intrapartally as proposed by Hammacher and Hon (10, 12) seems to be generally accepted. In intrapartum monitoring their interpretation has also been of fundamental importance. On this basis various scoring systems have been developed (4, 9, 14, 17, 19, 24). However in some studies so-called fetal heart reactivity has received most attention in the assessment of fetal condition (4, 6, 16, 20).

Whatever classification of the various CTG patterns might be chosen the main question must be whether antenatal CTG can be used as a reliable screening test for identifying the fetus at risk. In this respect one would be prepared to accept some cases of false pathologic tracings (over diagnosis). The occurrence of false normal tracings however is not what one would expect from a reliable screening test.

One essential aim of all antenatal care is to prevent fetal death and the purpose of the present work was to elucidate the reliability of antenatal CTG in predicting fetal death.

## MATERIAL AND METHODS

In the catchment area of the Department of Obstetrics and Gynecology of the University Hospital in Lund practically all pregnant women visit the antenatal clinics regularly during pregnancy. For various complications about 15 per cent of them are hospitalized for shorter or longer periods during the last part of the pregnancy. From March 1976 to March 1980 1899 patients were hospitalized on the antenatal ward. Apart from some exceptions during the first months of the period studied all patients which at least had reached the 28th week of gestation were monitored daily with CTG. Thus 1455 patients (76.6 per cent) have been routinely monitored with CTG. The mean number of diagrams per patient was 7-8 and the whole material therefore consists of more than 10000 cardiotocograms. In the whole material ultrasound fetal cardiology was generally used. Only in selected cases was abdominal fetal electrocardiography performed. In all 5 cases reported ultrasound fetal cardiology was used. Each CTG has been sorted according to the classification shown in Table 1. Only one criterion

Table 1 Classification of antenatal CTG

## Class 1 (Normal CTG)

Baseline 120-160 beats/min  
Variability (band width) > 10 beats  
No decelerations

## Class 2 (Suspect pathological CTG)

Baseline 100-120 or 160-180 beats/min  
Variability (band width) > 25 beats  
Variability (band width) 5-10 beats for less than 10 min  
No accelerations

## Class 3 (Slight pathological CTG)

Baseline < 100 or > 180 beats/min  
Variability (band width) 5-10 beats for more than 10 min  
Scattered moderate variable decelerations

## Class 4 (Severe pathological CTG)

Variability (band width) < 5 beats  
Severe variable decelerations  
Late decelerations



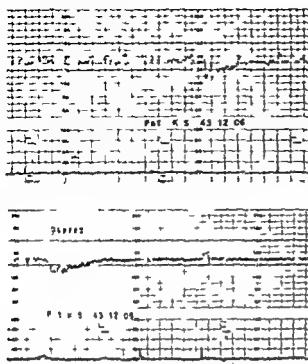


Fig 1 Antenatal CTG from case 1 demonstrating severe pathological changes. Upper and lower tracings show parts from the same recording. The frequency is about 130 beats per minute (bpm). The variability in the upper tracing seems normal but in the lower part the variability is clearly decreased. The externally registered tocogram does not reflect the uterine contractions very well but after two contractions late decelerations are induced.

was needed to refer the patient to one of the classes. During the first 2 years of the period the traces were judged by the authors. However, as the classification proved to be convenient and reliable (2), interpretation of the antenatal CTGs has since been performed by several members of the staff.

## RESULTS

Of the 1455 patients routinely monitored with CTG during the 4 year period, 5 cases of fetal death occurred (0.34 per cent). In each of these 5 cases pathological CTG patterns were seen on the traces.

**Case 1** A 33 year-old woman, gravida 3 para 2 was admitted in July 1976 in the 33rd week of gestation by reason of pre-eclampsia and intrauterine growth retardation (IUGR). CTG was performed 2-3 times per week. Sixteen days after admission the CTG showed severe pathological changes (Class 4) (Fig. 1). This CTG abnormality was overlooked and therefore no further CTG was done during the subsequent

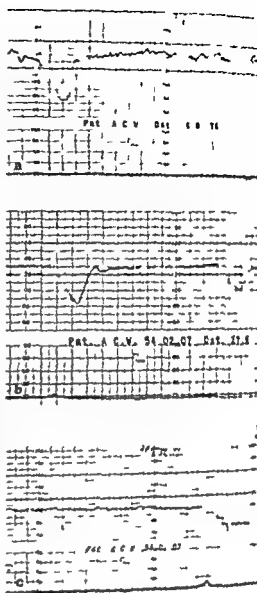


Fig 2 Antenatal CTG from case 2.

a) CTG trace on admission. The frequency is about 130 bpm. The variability shows silent pattern. No contractions, profound decelerations are present.  
b) CTG tracing one day after admission. The frequency is about 130 bpm, the variability shows a silent pattern. No contractions, profound decelerations occur one after another.  
c) CTG tracing 7 days after admission. The frequency is about 100 bpm, the variability shows a silent pattern.

days. Four days after the last CTG fetal death was diagnosed.

**Case 2** A 22 year-old woman, gravida 2 para 1 was admitted in the 31st week of gestation by reason of pre-eclampsia and IUGR. The sum of the

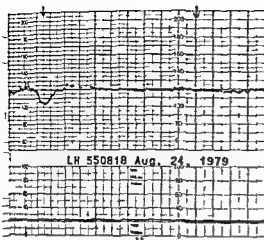


Fig. 3 Antenatal CTG from case 3. The frequency is about 120 bpm. Silent pattern and deceleration are present the day before fetal death. No contractions were visualized on the program.

responded to the 27th week of gestation and the fetus was not judged viable *ex utero*. Already on admission there were pathological CTG patterns occurred (Fig. 2 a). During the next 2 days an aggravation of the ominous pattern took place and the fetus died *in utero* (Figs 2 b c).

Case 3 A 24-year-old primigravida was admitted in the 29th week of gestation by reason of pre-eclampsia and IUGR. The size of the uterus corresponded to the 28th week of gestation. The fetus was not judged viable *ex utero*. On the day after admission a severe pathological CTG pattern was seen (Fig. 3). Two days after admission no fetal heart sounds could be detected.

Case 4 A 42-year-old woman gravida 2 para 3 was admitted in the 29th week of gestation by reason of pre-eclampsia. A twin pregnancy had earlier been diagnosed and the fetuses had been thoroughly examined by ultrasound scanning. IUGR had been diagnosed in one of the twins. CTG was normal on both twins during the first days (Fig. 4 a). After 8 days the IUGR twin showed a slight pathological CTG (Fig. 4 b). During the subsequent days the CTG pattern worsened (Figs 4 c d). Fourteen days after admission the IUGR twin showed no signs of life. Throughout the period the other twin showed a normal CTG and both the dead and the living twin were delivered in the 39th week of pregnancy.

Case 5 A 37-year-old woman gravida 4 para 2 was admitted in the 33rd week of gestation by reason of pre-eclampsia and IUGR. After a day the first slight pathological CTG occurred (Fig. 5 a). After 2 days the pattern worsened (Fig. 5 b) and an oxytocin stress test (OST) was performed which proved clearly positive (Fig. 5 c). It was decided to administer cortisone for lung maturation and the next day the CTG was normal (Fig. 5 d). On the fifth day repeated ominous CTGs occurred (Fig. 5 e). Caesarean section was decided on later the same day but the child was stillborn.

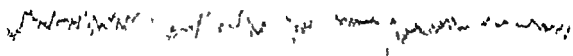
## DISCUSSION

Various methods exist for the supervision of mother and fetus during pregnancy. For instance blood pressure and symphysis fundal measurements are used routinely in all pregnancies to estimate the progress of pregnancy. Despite this the existing methods have been considered inadequate and consequently search has been made for a reliable method that could be used in all pregnancies for detection of the fetus at risk. A perfect screening method in this respect must be convenient for both the patient and the personnel. It should be neither too expensive nor too time-consuming. The results must always be unequivocal, easy to interpret and no false normal results should occur. Does antenatal CTG fulfil these criteria? The answer is no because CTG is time-consuming and places considerable demands on the staff and is therefore expensive. For personnel who are not specially trained it can be difficult to arrive at a correct interpretation. In order to discover the risk cases during pregnancy continued use of the routine methods combined with ultrasound scanning is preferable at the antenatal clinics.

In our catchment area about 15 per cent of all pregnant women have been hospitalized because of pathologic findings at routine examination (including ultrasound scanning) at the antenatal clinics or because of acute incidents such as bleeding and premature contractions. In a previous report we stated that about 40 per cent of these patients could be classified as high risk patients (22). Nearly all the pathologic antenatal CTGs were found in this group. The conclusion was therefore that primarily high risk patients should be routinely monitored with antenatal CTG. In the present study this conclusion was confirmed by the fact that all cases of intrauterine death had the diagnosis pre-eclampsia and IUGR.

TWIN I

TWIN II



E I MW 480516 Dec 30 1979

G II MW 480516 Dec 30, 1979

X

X



TWIN I

TWIN II



E I MW 480516 Jan 5 -80

G II MW 480516 Jan 5 1980

X

X

Fig 4 Antenatal CTG from case 4

a) One day after admission both twin I and twin II show normal CTGs

b) Nine days after admission Twin I shows normal CTG. The CTG from twin II demonstrates a fetal heart rate of 120 bpm and one deceleration of variable pattern

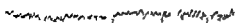
As intrauterine death so seldom occurs it is necessary to study a large number of patients in order to evaluate the reliability of CTG monitoring in predicting these cases. In the present investigation more than 1400 patients and 10000 CTGs were studied. Five intrauterine deaths occurred during the observation period. In each of these cases pathologic changes in the CTG pattern predicted the outcome.

Since the early description by Hon of changes in fetal heart rate preceding fetal death (11) many

reports have been published on the subject. Kubli *et al* (15) reviewed 13 cases of intrauterine death. In all but one case pathologic CTGs of the same nature as in the present report were found. The same case had no antenatal CTG done. In the present case the CTG done 48 hours prior to intrauterine death is evidence of the reliability of CTG in predicting fetal death. This has also been stressed (3, 7, 8, 21).

Although fetal death can occur without pathologic changes this seems to be extremely rare (21). An

TWIN I



G I MV 516 Jan 7 1980

X



TWIN II



G II PV 480516 Jan 7 1980

X



Two days after admission Twin I shows normal CTG  
CTG from twin II demonstrates silent pattern and  
decelerations of sinusoid appearance

TWIN I



G I MV 480516 Jan 10 1980

X



TWIN II



G II PV 480516 Jan 10 1980

X



d) Thirteen days after admission (less than 74 hours before  
fetal death) Twin I shows normal CTG The CTG from  
twin II shows silent pattern and decelerations

long our own patients and also in the case reported  
Hubli (13) the explanation could be too long an in-  
terval between registrations. It would therefore seem  
justified to conclude that the intervals should not ex-  
ceed 2 days. In cases of umbilical cord complications  
which may occur antenatally and *ablatio placentae*

the ability of CTG to predict fetal death might not be  
sufficiently reliable. However van Wering *et al* (25)  
published 4 cases of *ablatio placentae* where CTG  
warned of fetal distress.

As mentioned in the introduction one would be  
prepared to accept some cases of false pathologic



RG 437202 Jan 10 1980



a) Antenatal CTG from case 5  
One day after admission. The frequency is 125-130  
b, decreased variability and one deceleration



RG 437090 Jan 12 1980



b) Three days after admission. Repeated decelerations of  
uncertain nature (no contraction visible)

ME 430902 Jan 12 1980 CST

PG 43 9 2 JA 12 113

c) Oxytocin stress test 3 days after admission. Decreased variability and repeated late decelerations after each contraction

d) Four days after admission and after correction of anemia. Normal CTG is registered

CTG (over diagnosis) provided all fetuses at risk could be identified. In the present study 5 cases of intrauterine death among 1455 CTG monitored patients were retrospectively examined concerning the CTG patterns preceding fetal death. In all 5 cases severe pathological CTG patterns were found. During the investigated period approximately 50–60 more patients (4 per cent) showed severe pathological CTG patterns (22). These patients were all delivered within a few hours after the appearance of such patterns. The proportion of over diagnosis among these cases cannot be evaluated. The results of the present study together with reports from other authors support the view that prompt action must be taken to deliver a fetus showing severe pathological CTG patterns. Although the proportion of overdiagnosis cannot be enumerated exactly, CTG must be considered a most reliable method of predicting fetal death.

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e) Five days after admission (same day as fetal death occurred). Decreased variability and one deceleration probably of late nature

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Submitted for publication September 4 1980

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# MATERNAL FETAL AND NEONATAL EFFECTS OF BETA ADRENERGIC STIMULATION IN CONNECTION WITH CESAREAN SECTION

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**Summary** To investigate the combined metabolic effects of beta-mimetic therapy and general anesthesia on maternal and fetal/neonatal metabolism 14 patients were treated with isotonic saline 11 with intravenous fenoterol (3 µg/min) and 9 with intravenous isoxsuprine (150 µg/min) for 30 min prior to cesarean section. The maternal heart rate and blood pressure as well as the maternal and fetal acid-base balance were followed. The neonatal glucose level was measured for 36 hours after delivery.

The heart rate and the diastolic and systolic blood pressure values increased during the operation in each group without any marked differences between the groups. At the end of the operation the mean maternal BD (base deficit) value was higher in the fenoterol patients than in the control patients, indicating a trend towards metabolic acidosis. The other values of acid-base balance in the maternal and umbilical arterial and venous blood did not reveal any differences between the groups.

The neonatal glucose level at 2 hours after delivery was higher in the fenoterol group than in the control group. The other values recorded during 36 hours indicated no differences between the groups.

Beta-mimetic treatment preceding general anesthesia in cesarean section does not have unfavorable effects on the mother, the fetus or the newborn. Such therapy thus does not seem contraindicated when uterine contractions should be artificially suppressed in cases of fetal distress before operation.

Beta-sympathomimetic drugs have been used increasingly in obstetric practice to suppress uterine contractions (8, 16, 17). It has also been shown that fetal distress and acidosis can be successfully treated with beta-sympathomimetics (3, 7, 10), possibly by improving uteroplacental circulation (17).

Cesarean section involves stress factors which may be those of vaginal delivery deteriorate the uteroplacental circulation and affect the fetus and the newborn infant, as shown by the Apgar scores, the acid-base values and the neonatal morbidity and mortality (2, 15). The condition of the newborn in the first 24 hours after cesarean section correlates possibly best to

the placental perfusion during the anesthesia induction — delivery interval (9, 12). It would thus be ideal to maintain the placental blood flow as well as possible during this time. The beta-sympathomimetic drugs could theoretically be of value in this respect.

The purpose of this study was to elucidate the effects of preoperative intravenous infusion of fenoterol and isoxsuprine on maternal, fetal and neonatal metabolism and maternal circulation in connection with elective cesarean section performed under general anesthesia in normal pregnancies.

## MATERIAL AND METHODS

The series consisted of 34 healthy mothers in the 38th—43rd week of normal pregnancy (Table 1). The indication for elective cesarean section was a fetopelvic disproportion in 29 cases and previous cesarean sections in 5 cases. None of the mothers had uterine contractions prior to the study. The study and its purpose were explained to the mothers and their consent was obtained.

At 8:00 a.m. an i.v. infusion of isotonic saline (14 cases control group), fenoterol (11 cases) or isoxsuprine (9 cases) was started and maintained for 30 minutes. The infusion rate for fenoterol was 3 µg/min and that for isoxsuprine 150 µg/min. General anesthesia was induced 10 minutes after the cessation of infusion.

Table 1 Age, parity and gestational weeks of the parturients examined

Group		Age	Parity	Gestation in weeks
Control group (n=14)	mean	29.0	2.0	40.2
	range	19–38	1–3	39–43
Fenoterol group (n=11)	mean	27.3	1.9	39.9
	range	18–43	1–5	38–42
Isoxsuprine group (n=9)	mean	26.1	1.8	40.0
	range	19–30	1–3	39–41



Table II The mean ( $\pm$ SD) induction doses of thiopental induction—delivery interval (I—D time) and blood losses during cesarean section

Group	Doses of thiopentone			Blood loss
	total (mg)	mg/kg	I—D time (ml)	
Control group (n=14)	277 $\pm$ 31	4.2 $\pm$ 0.8	8.35 $\pm$ 2.30	981 $\pm$ 361
Fenoterol group (n=11)	300 $\pm$ 35	4.4 $\pm$ 0.4	7.49 $\pm$ 2.17	894 $\pm$ 217
Isosuprine group (n=9)	278 $\pm$ 51	4.1 $\pm$ 0.8	7.40 $\pm$ 1.13	789 $\pm$ 359

The patients were unpremedicated. Before the induction 0.5 mg atropine i.v. was given. Oxygen was administered by 10 l/min at 3 minutes after which thiopental for the induction was given (Table II) and endotracheal intubation was completed with the aid of succinylcholine (3 mg/kg). Anesthesia was maintained with a mixture of fentanyl and  $N_2O$  and succinylcholine infusion. The patient was positioned on the operation table as at least 15° left lateral to avoid aortocaval compression. A femoral artery cannula and blood pressure were recorded every five minutes during the infusion and the operation at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, 285, 300, 315, 330, 345, 360, 375, 390, 405, 420, 435, 450, 465, 480, 495, 510, 525, 540, 555, 570, 585, 600, 615, 630, 645, 660, 675, 690, 705, 720, 735, 750, 765, 780, 795, 810, 825, 840, 855, 870, 885, 900, 915, 930, 945, 960, 975, 990, 1005, 1020, 1035, 1050, 1065, 1080, 1095, 1110, 1125, 1140, 1155, 1170, 1185, 1200, 1215, 1230, 1245, 1260, 1275, 1290, 1305, 1320, 1335, 1350, 1365, 1380, 1395, 1410, 1425, 1440, 1455, 1470, 1485, 1500, 1515, 1530, 1545, 1560, 1575, 1590, 1605, 1620, 1635, 1650, 1665, 1680, 1695, 1710, 1725, 1740, 1755, 1770, 1785, 1800, 1815, 1830, 1845, 1860, 1875, 1890, 1905, 1920, 1935, 1950, 1965, 1980, 1995, 2010, 2025, 2040, 2055, 2070, 2085, 2100, 2115, 2130, 2145, 2160, 2175, 2190, 2205, 2220, 2235, 2250, 2265, 2280, 2295, 2310, 2325, 2340, 2355, 2370, 2385, 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Table V Maternal arterial, umbilical arterial and venous blood pH,  $P_{CO_2}$  (kpc),  $PO_2$  (kpc) and BD (mmol/l) means  $\pm$  SD

Group	Maternal arterial before anesthesia	Maternal arterial at delivery	Umbilical venous	Umbilical arterial
Control				
pH	7.43 $\pm$ 0.04	7.35 $\pm$ 0.04	7.30 $\pm$ 0.04	7.26 $\pm$ 0.04
$P_{CO_2}$	3.4 $\pm$ 0.6	4.6 $\pm$ 0.4	5.8 $\pm$ 0.5	4.4 $\pm$ 0.6
BD	5.2 $\pm$ 2.0	5.9 $\pm$ 1.1	5.0 $\pm$ 1.5	5.8 $\pm$ 2.2
$PO_2$	14.5 $\pm$ 0.8	22.9 $\pm$ 3.3	5.2 $\pm$ 1.1	3.2 $\pm$ 0.7
Fenoterol				
pH	7.37 $\pm$ 0.03	7.33 $\pm$ 0.04	7.30 $\pm$ 0.03	7.26 $\pm$ 0.04
$P_{CO_2}$	3.9 $\pm$ 0.5	4.4 $\pm$ 0.4	5.8 $\pm$ 0.2	7.1 $\pm$ 0.9
BD	6.9 $\pm$ 0.8	7.5 $\pm$ 1.5	5.2 $\pm$ 2.0	4.4 $\pm$ 2.3
$PO_2$	14.7 $\pm$ 2.4	23.6 $\pm$ 5.6	5.1 $\pm$ 1.6	2.6 $\pm$ 0.5
Isoxsuprine				
pH	7.39 $\pm$ 0.03	7.34 $\pm$ 0.04	7.29 $\pm$ 0.02	7.26 $\pm$ 0.02
$P_{CO_2}$	3.8 $\pm$ 0.1	4.4 $\pm$ 0.3	5.9 $\pm$ 0.2	6.5 $\pm$ 0.2
BD	6.7 $\pm$ 1.2	6.8 $\pm$ 1.3	5.4 $\pm$ 1.0	4.2 $\pm$ 2.0
$PO_2$	16.0 $\pm$ 1.5	22.9 $\pm$ 6.1	5.3 $\pm$ 0.6	3.7 $\pm$ 1.4

p &lt; 0.05 compared with the control group

The newborns were in good condition after delivery. The Apgar scores at 1 min and 5 min did not differ between the groups (Table III). One baby in the fenoterol group received 6 points at 1 min but 9 points at 5 min.

**Maternal heart rate and blood pressure** The lowest heart rate before the induction of anesthesia was seen in the control group (Table IV). It did not differ significantly from the corresponding values in the other groups. In all of the groups the heart rate increased during the anesthesia and the operation. In the isoxsuprine group the mean heart rate at the beginning of the operation was significantly higher than the initial value ( $p < 0.01$ ) or the corresponding value in the control group ( $p < 0.01$ ). At the moment of delivery the mean heart rates in the fenoterol and the isoxsuprine groups were significantly higher than in the control patients ( $p < 0.05$ ).

Table VI Blood glucose (mmol/l) of newborn infants in cesarean section: means  $\pm$  SD

Group	2 hours	6 hours	11 hours	24 hours	36 hours
Control	2.7 $\pm$ 0.4	2.7 $\pm$ 0.8	2.8 $\pm$ 0.8	2.5 $\pm$ 0.7	2.6 $\pm$ 0.7
Fenoterol	3.3 $\pm$ 0.3	2.7 $\pm$ 0.4	2.9 $\pm$ 0.4	3.0 $\pm$ 0.7	3.3 $\pm$ 0.1
Isoxsuprine	2.6 $\pm$ 0.6	2.8 $\pm$ 0.4	2.9 $\pm$ 0.5	2.8 $\pm$ 0.5	2.7 $\pm$ 0.6

p &lt; 0.05 compared with the control group

Before the induction of anesthesia the lowest mean systolic blood pressure was recorded in the isoxsuprine group (Table IV). This value differed significantly from that in the fenoterol group ( $p < 0.05$ ). At the other times there were no statistically significant inter group differences in the systolic blood pressures. In all the groups the mean systolic blood pressure increased significantly during the anesthesia and the operation. In the fenoterol and the isoxsuprine groups this increase was more rapid than in the control group.

The lowest mean initial diastolic pressure was seen in the isoxsuprine group (Table IV). This value differed significantly from that in the control group ( $p < 0.05$ ). The mean diastolic pressure also increased during the operation. The mean values recorded at delivery were significantly higher than the mean initial values in all the groups ( $p < 0.01$ ).

The mean pulse pressure did not change significantly during the operation in any group. At delivery the mean pulse pressure in the isoxsuprine group was significantly lower than in the fenoterol group ( $p < 0.05$ ).

**Maternal and fetal acid base balance** Before the induction of anesthesia there were no significant inter group differences in the maternal arterial acid base values or  $PO_2$ . At the moment of delivery these values were at the same level as initially. At that time however the mean maternal base-deficit (BD) in the fenoterol group was significantly higher than in the control group ( $p < 0.05$ ) (Table V).

The umbilical arterial and venous acid base values and  $PO_2$  did not differ significantly between the groups (Table V).

**Neonatal glucose.** At the age of 2 hours the mean neonatal glucose of the fenoterol group was significantly higher than that of the control group ( $p < 0.05$ ) (Table VI). At the other times there were no significant differences between the groups. The decrease between 2 and 6 hours after delivery in the fenoterol group was significant ( $p < 0.05$ ) while at the other times the glucose values did not differ from the 2 hour level in any group.

## DISCUSSION

During the induction of general anesthesia for cesarean section the placental blood flow has been shown to decrease (12). In connection with an elective section in normal pregnancies however this seems to be of no great clinical importance. The newborns are generally in good condition (9-12). The fetal distress caused by uterine contractions may, however, be increased because of the anesthesia. In such cases pretreatment with beta mimetics might therefore be beneficial and improve the fetal state. However it is important to study first the effects of combined beta mimetics and anesthesia.

The maternal cardiovascular effects of beta mimetic drugs are tachycardia and hypotension (20). Especially a fall in the diastolic blood pressure and a widening of the pulse pressure have been emphasized. The cardiovascular and metabolic effects of the beta adrenergic drugs have been shown to become evident in a few minutes (6). The present findings concerning the maternal cardiovascular effects of fenoterol and isoxsuprine used in tocolytic concentrations are in accordance with the previous reports. The lowest initial blood pressure and the highest pulse rate were observed in isoxsuprine parturients. It is known that isoxsuprine in tocolytic concentrations has a more profound cardiovascular action than fenoterol (6). The patients in the fenoterol and isoxsuprine groups responded to the anesthesia and the operation more markedly than the controls. This was evidenced as a greater rise of blood pressure in the beta mimetic patients. The present healthy mothers had no controversial effects of this. In patients with cardiovascular diseases however the beta mimetics should be avoided because of the risk of an incipient cardiac decompensation which has been shown to occur after high doses of beta mimetics (11).

The main maternal metabolic changes at the beginning of an infusion of beta mimetic drugs are lipolysis and glycogenolysis at a constant rate immediately (13-20). The changes in the acid base balance which have been shown in the acute metabolic acidosis are secondary to the primary changes. In the present study the acid base parameters also showed a metabolic acidosis in the fenoterol group but a higher BD values than in the control group were detected.

An ideal placental blood flow is the primary factor for fetal well being. During the general anesthesia for cesarean section there are factors which enhance placental perfusion (12). Beta stimulation causes vasodilatation together with a widened pulse and an increased cardiac output can be expected to enhance placental perfusion (21). There are reports which have shown beta mimetics to have beneficial effects on intrapartum fetal acidosis (17) and fetal capillary blood  $PO_2$  values have also been reported during an infusion of fenoterol (18). In the present cases of normal pregnancy a beta mimetic of placental disturbances and without uterine contractions the beta mimetics do not seem to have any remarkable effects on the mother or the newborn as evidenced in the acid base parameters or the Apgar scores. In cases of an induced and uterine contractions these findings could be different.

During beta mimetic tocolysis there are glucose metabolism also occurs in the fetus (9). The sympathetic action appears immediately after starting intravenous application of sympathomimetics (11). On the other hand the previous results have shown that blood glucose values of the fetus, the newborn and the mother at birth correlate with each other (18). The lowest neonatal glucose levels have been reported at the age of two hours (5, 11). It is therefore before the first neonatal blood glucose levels were assayed at this time. They showed no fetal hypoglycemia in any of the babies. In the fenoterol group the mean neonatal blood glucose at 2 hours was significantly higher than in the control babies which reflects the hyperglycemic action of the drug. This finding does not support the suggestion that during an acute tocolytic therapy the fetal glycogen stores are depleted and fetal hyperglycemia occurs in the newborn (20). According to our results the present short term tocolytic therapy does not seem to have any harmful effects on neonatal glucose metabolism.

The findings of this study show that general anesthesia and beta mimetics in healthy parturients not have unfavorable combined effects on the mother, the fetus or the newborn. In complicated pregnancies fetal distress may be caused or at least exacerbated by uterine contractions which diminish the placental blood flow. In such cases the delivery often terminated with cesarean section. The attempt to obtain an immediate uterine relaxation with intravenous infusion of a beta mimetic drug prior to operation seems in the light of the present data to be in no way contraindicated.

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Submitted for publication December 12 1978

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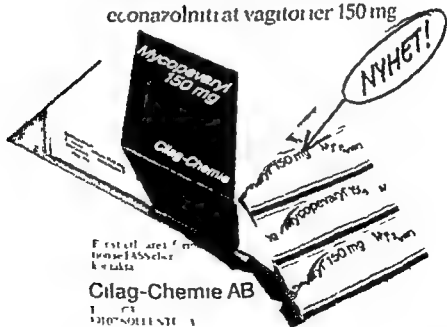


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# MATERNAL ALCOHOL AND TOBACCO USE AND NAUSEA AND VOMITING DURING PREGNANCY

Relation to infant birthweight

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**Abstract.** A recent report has documented a decreased probability of nausea and vomiting during pregnancy (NVP) in women who were simultaneously both regular drinkers and smokers, especially if these habits existed before pregnancy. Maternal smoking and drinking have both been linked with decreased infant birthweight, as has absence of NVP. The question posed by these findings is whether the decrease in birthweight associated with absence of NVP is in fact due to maternal drinking and smoking. The data presented in this paper indicate that this is not the case. NVP is related to infant birthweight even after adjustment for maternal alcohol and tobacco use.

The association of nausea and vomiting during pregnancy (NVP) with favorable pregnancy outcome has frequently been reported. Risk of abortion prior to the 16th week of pregnancy is decreased if NVP is present (2, 4, 7, 9) and live born children are reported to be of greater gestational age and higher birthweight (7).

However, a recent report has linked probability of NVP with maternal alcohol and tobacco use (6). NVP was reported less frequently by women who were simultaneously both regular drinkers and smokers, especially if these habits existed prior to pregnancy. Maternal drinking and smoking have both been related to decreased infant birthweight (1, 3, 5, 8). The purpose of this paper is to determine if presence of NVP is related to higher birthweight when maternal drinking and smoking habits and their relationship to NVP are taken into account.

## SUBJECTS AND METHODS

Subjects and methods in this investigation have been described in detail elsewhere (6). Briefly, a consecutive sample of 106 prenatal patients in a large health maintenance organization were screened for alcohol and tobacco use.

The first 162 subjects plus all women drinking an average of at least 15 ml of absolute alcohol daily before pregnancy or in early pregnancy were studied ( $n = 222$ ). Of these, all women who bore single live children are included in this report ( $n = 199$ ). An in-depth interview in the fourth month of pregnancy provided information on drinking since conception and in the 6 months prior to it, as well as data regarding smoking, beverage consumption, and drug use. Alcohol use was expressed in average milliliters of ethanol consumed daily (AA Score). Regular drinking was defined to be an AA score of 0.5 or more (equivalent to at least 30 ml of 100-proof whiskey daily). AA scores of 0.1 or less were classified as infrequent drinking, with intermediate AA scores defined as occasional drinking. Smokers lit an average of at least one cigarette per day. Occurrence of NVP refers to nausea and vomiting in the first four months of pregnancy as reported by the subject.

## RESULTS

The initial analysis deals with drinking in the 6 months before pregnancy. Table 1 shows mean infant birthweight by maternal smoking, drinking, and NVP status. In general, birthweight of infants was less if NVP was absent, regardless of alcohol or tobacco use. Other factors which might influence birthweight were then considered. Table 2 shows mean maternal height, age, parity, gestational age, and per cent of male children in the groups. Maternal age and sex of child were considered to be possible confounding variables. These, as well as AA score, smoking, and NVP status, were regressed on birthweight. Only AA score and NVP contributed significantly to the variance. The equation was then recomputed with only the three primary independent variables. The result is shown in Table 3. Addition of interaction terms failed to change the significance of NVP.

The identical analysis was then repeated for early pregnancy drinking and smoking. Again, NVP was significantly related to infant birthweight even when

Table 1 Mean birthweight of infants (gm) by maternal drinking smoking and NVP status (N=17)

	Occasional or infrequent drinking		Regular drinking	
	Nonsmoker	Smoker	Nonsmoker	Smoker
Before pregnancy				
NVP	3 622 ( 66)	3 521 (15)	3 583 (46)	3 473 (16)
No NVP	3 474 ( 20)	3 475 ( 7)	3 468 (11)	3 380 (13)
Early pregnancy				
NVP	3 631 (102)	3 437 (18)	3 489 (15)	3 359 ( 8)
No NVP	3 440 ( 29)	3 394 (14)	3 355 ( 4)	3 168 ( 9)

Number in subgroup shown in parentheses beside mean birthweight of subgroup

controlling for alcohol and tobacco use in early pregnancy Table 1 indicates that absence of NVP is linked to lower birthweight regardless of alcohol and tobacco use. Regression of the three primary variables on birthweight (Table 3) confirms this observation. Regression results are altered if number of cigarettes (rather than binary smoking status) is entered as the tobacco use variable during pregnancy; then number of cigarettes contributes significantly to birthweight variance as does NVP.

### DISCUSSION AND SUMMARY

The hypothesis that the beneficial effect of NVP on birthweight is due to infrequent drinking and smok-

ing among women with NVP is not supported by data presented here. In fact, alcohol use (prior to pregnancy only), smoking (during pregnancy and NVP) each appear to be significantly correlated with infant birthweight. According to these data, NVP may still be considered a favorable prognostic sign and not the reflection of abstaining from use of tobacco and alcohol.

### ACKNOWLEDGEMENT

This research was supported by grant No. AA-00044 from the National Institute on Alcohol Abuse and Alcoholism and by the Alcoholism and Drug Abuse Center at the University of Washington.

Table 11 Selected factors influencing birthweight by maternal drinking before pregnancy NVP and smoking status

	Occasional or infrequent drinking		Regular drinking	
	Nonsmoker	Smoker	Nonsmoker	Smoker
Maternal height (cm)				
NVP	163	165	163	164
No NVP	163	165	163	165
Maternal age (years)				
NVP	26	26	29	27
No NVP	27	21	28	7
Parity				
NVP	1	1	1	1
No NVP	1	1	1	1
Gestational age (weeks)				
NVP	40	40	40	40
No NVP	40	40	40	39
Sex of child (male in per cent)				
NVP	59	44	41	46
No NVP	40	71	73	61

Table III Regression of AA score NVP and smoking status on infant birthweight

Variable	b	SE	R <sup>2</sup>
AA score before pregnancy	-82.4	02	046
NVP <sup>1</sup>	148.8	05	072
Smoking status before pregnancy <sup>2</sup>	-127.8	10	085
AA score in early pregnancy	-102.3	31	019
NVP <sup>1</sup>	153.3	04	048
Smoking status in pregnancy	-151.2	07	064

b = regression coefficient (slope) R<sup>2</sup> = proportion of variance explained by this and all preceding variables in the equation  
 P = probability of significance of b taking account of other variables in the equation

01 No NVP      Some NVP

00 Nonsmoker    Smoker

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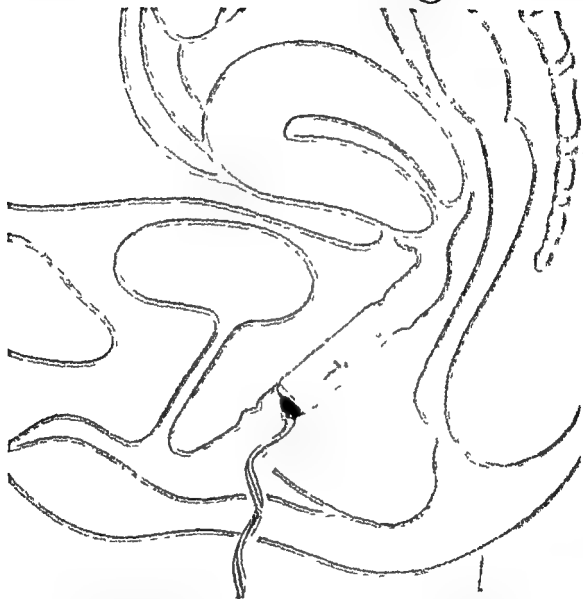
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Submitted for publication September 6 1979

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## URINARY FIBRINOLYSIS IN TOXEMIA OF PREGNANCY

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**Abstract.** Serial determinations of urinary plasmin level by a dodecascapolytic assay were performed in 40 healthy women and 79 patients with various types of toxemia throughout pregnancy, labor and the puerperium. The results indicate that:

1. The urinary plasmin level in normal pregnancy increased gradually from the third trimester to the early puerperium.

2. In mild pre-eclampsia, a significant rise in urinary plasmin level from 36 weeks of gestation to the 4th day postpartum.

3. In severe pre-eclampsia, a progressive decrease in late pregnancy was found. After delivery in the early puerperium, the urinary plasmin level of the patients with a rapid onset was significantly higher than that of the patients with the sequelae of toxemia.

Our study showed that serial assays of urinary plasmin may provide a reliable and sensitive index of severity, progression and natural history of toxemia of pregnancy.

The term "toxemia of pregnancy" has been applied to the occurrence of various combinations of hypertension, albuminuria and edema, sometimes further complicated by the development of convulsions and coma, occurring after 20 weeks of gestation. These various lesions make up a confusive complex of related syndromes, the etiologies of which have remained obscure despite extensive investigation along many lines. The conflict of opinions is not limited to the question of etiology but extends to the practical problem of clinical management.

The work of groups under McKay, Vassalli, and Under Sheehan and others suggests that toxemia is related to a continuing slow state of intravascular coagulation which produces a deposition of fibrin or fibrin-like material in the glomeruli (1-9, 11-14, 15, 16, 17). In eclampsia the process is massive and leads to thrombosis of capillary vessels, which is visible by light microscopy. In pre-eclampsia the clotting is slow and incomplete but progressive and does not lead to the occlusion of

capillaries but to deposits of macromolecular aggregates of fibrin on the capillary basement membrane which can only be visualized by electron microscopy and immunohistochemical techniques (10, 12). McKay and Corey demonstrated that in pre-eclampsia the amount of circulating cryofibrinogen was greater than during normal pregnancy, labor, delivery and the puerperium (7). De Bacalao and Sedlis suggested that an increased platelet adhesiveness in toxemia promoted aggregation and destruction of the platelets in the placenta and other organs (8).

Attention has recently been focused on the fibrin/fibrinogen degradation products (FDP) in blood and urine in patients with toxemia, such products being considered a sign of active pathological processes (2, 3). But despite much attention to these problems little is known about the urinary excretion of plasminogen and plasmin (urinary PLS) in normal pregnancy and toxemia. These may reflect more closely the extent of intraglomerular fibrinolysis in the pathogenesis of pre-eclampsia and eclampsia.

In the present study, serial sensitive and quantitative assays of plasminogen and plasmin have been used for the detection of intraglomerular fibrinolysis in groups of healthy and toxemic women throughout pregnancy, labor and the puerperium.

## MATERIAL AND METHODS

Forty healthy pregnant women were studied serially from the first trimester to term and during labor and the puerperium with their full informed consent. In addition 15 patients with mild pre-eclampsia, 7 with severe pre-eclampsia, four with abruptio and three with eclampsia were studied. Thirty-seven healthy non-pregnant women ranging from 20 to 30 years of age (mean 24.4 years) and receiving no form of medication were used as controls.

For prevention of contaminations from exogenous sources such as blood or discharges from birth canal to compound diagnostic problems, urine samples were obtained by catheterization and prophylactic antibiotics were given to all patients studied.

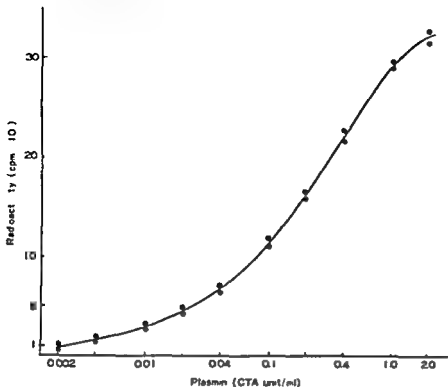


Fig 1 Standard curve of lysine Sepharose affinity chromatography radioimmunoassay for plasmin

The urine samples were centrifuged at 3 000 r.p.m. at 4 °C and the supernatant was adjusted to pH 7–7.5 and then assayed immediately or stored in aliquots in plastic tubes at –40 °C until use.

**Solid state radioimmunoassay for plasminogen and plasmin antigenicity.** Anti human plasminogen rabbit serum was prepared by the methods described by Robbins and the IgG fractions were separated by saturated ammonium sulfate solution and chromatography on a DEAE cellulose column (13). The antiserum had the same binding capacity to plasmin and plasminogen. Anti plasminogen was labelled with  $^{125}$ I by a method described by Hunter and Greenwood and

preparations were obtained with a specific radioactivity of 5  $\mu$ Ci per microgram of protein (4). The assay was done by microtitre plate technique and gave an accuracy of 1% (14). As little as 10  $\mu$ g/l of plasminogen and plasmin can be detected by this method.

**Lysine Sepharose affinity chromatography radioimmunoassay for plasminogen and plasmin antigenicity.** A microcolumn containing 2 ml mixture of lysine Sepharose and  $^{125}$ I-labelled casein Sepharose was equilibrated with 0.05 M PIP at pH 7.4. Standard plasmin or urine samples were added to the microcolumn, followed by washing with 0.05 M PIP two times to remove urinary plasminogen and plasmin.

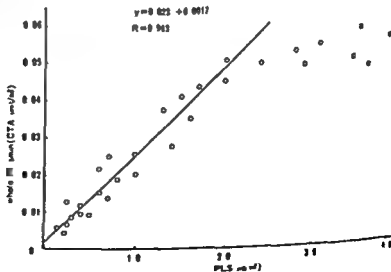


Fig 2 Relation between urinary PLS and whole plasmin in toxemia of pregnancy

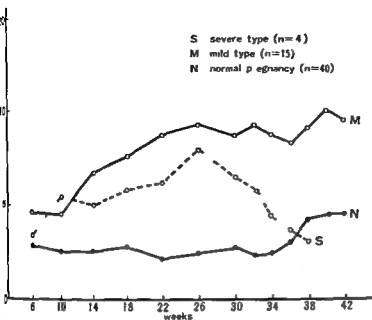


Fig 3 Sequential urinary plasmin studies in normal and toxemia of pregnancy during pregnancy

inhibitors. The column was then incubated at 37 °C for 90 min to digest casein by plasmin affixed on lysine Sepharose, and to release free  $^{125}$ I. The free  $^{125}$ I obtained was counted for radioactivity. The value obtained represented the free plasmin activity in urine. In order to measure the whole plasmin activity plasminogen in the samples was activated to plasmin by adding optimal amounts of urokinase to the microcolumn. The urinary plasmin was defined as the urinary total fibrinolytic activity expressed as plasmin, one part of which was already present in the urine and the other of which was converted in the column by urokinase from urinary plasminogen. The sensitivity of the assay method was 0.002 CTA unit/ml of plasmin (Fig 1).

## RESULTS

Plasminogen and plasmin in 33 urine samples from pre-eclamptic patients were measured by using solid phase radioimmunoassay and lysine Sepharose affinity chromatography radiocaseinolytic assay in order to correlate the two methods. As shown in Fig 2 there was a linear correlation between radioimmunoassay and radiocaseinolytic assay ( $Y = 0.023X + 0.0017$ ,  $r = 0.963$ ) in a concentration of less than 2 µg/ml plasminogen standard.

The results of the assay for plasmin in the urine of women during normal pregnancy, labor and the puerperium are shown in Fig 3 and 4. The urinary plasmin level of  $2.7 \pm 0.8$  CTA unit  $\times 10^{-3}$ /ml (mean  $\pm$  SD) in early pregnancy was slightly higher

than that of  $2.1 \pm 0.6$  found in control groups of non pregnant women. No significant alteration of the level took place during the course of normal pregnancy until the third trimester when the mean level rose to  $4.9 \pm 1.1$ , a significant increase ( $p < 0.05$ ) compared with that in early pregnancy. A further increase in plasmin level was found 4 hours before delivery. The level reached its peak at 4 hours postpartum and by the fourth day after delivery the mean plasmin level returned to the normal range for nonpregnant women.

As shown in Fig 3 urinary plasmin excretion in mild toxemia was significantly higher than in the corresponding stage of normal pregnancy ( $p < 0.005$ ) as early as the fourth month of pregnancy. The increase in plasmin level persisted during labor and the early puerperium (Fig 4) at a significantly higher level ( $p < 0.05$ ) than with a normal delivery group. But at the 5th postnatal day plasmin level was in the normal range.

In severe pre-eclampsia urinary fibrinolytic activity was elevated in early pregnancy. But by the end of the second trimester after the disease had been uncontrolled there was a progressive decrease in the mean value of plasmin. Near term the urinary plasmin level fell to a level lower than in the corresponding stages of normal pregnancy (Fig 3).

The severe cases of pre-eclampsia were divided into two groups on the basis of the postpartal clinical

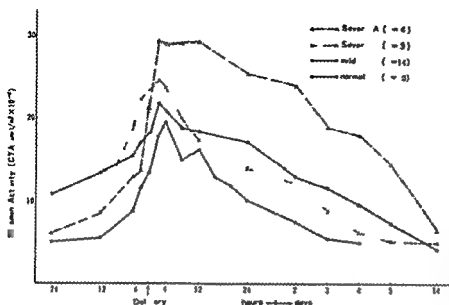


Fig 4 Sequential urinary plasmin studies in normotensive pregnancy, labor and the puerperium

course and the changes in urinary plasmin level (Fig 4). All the patients (4 cases) who showed so rapid a clinical improvement as to become normotensive and free from proteinuria within one month after delivery were classified into Group A. A striking rise of urinary plasmin level exceeding that of the normal delivery group ( $p < 0.001$ ) occurred within 4 to 12 hours postpartum, persisted virtually during the first week of puerperium, and then decreased gradually during the second week postpartum. By the end of the second week urinary plasmin level recovered to a normal or nearly normal value.

On the other hand, the 3 patients who had sequelae of proteinuria and/or hypertension one month after delivery were classified into Group B. A urinary plasmin level of Group B was slightly elevated during the first few days following delivery, but no statistical difference ( $p < 0.05$ ) in comparison with the normal puerperal subjects.

In abruptio placentae, urinary plasmin level fell strikingly within 24 hours after symptoms appeared, but after delivery the level sharply increased, indicating that the free plasmin was quickly eliminated from the circulation (Fig 5).

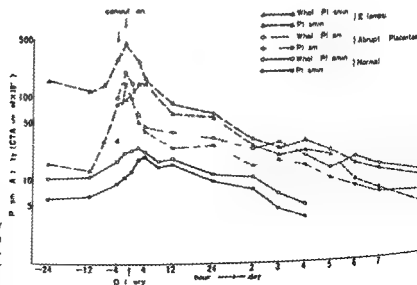


Fig 5 Sequential urinary whole plasmin and plasmin studies in eclampsia and abruptio placentae during labor and the puerperium

In three patients with eclampsia in late pregnancy the whole plasmin in urine increased steeply immediately after the eclamptic seizure of which only a very small proportion was shown to be free plasmin activity indicating that urinary fibrinolytic activity was diminished. But a pronounced increase in urinary free plasmin similar to the pattern in the case of abruptio placentae was observed after delivery in the patients with eclampsia.

## DISCUSSION

Our observations suggest that urinary plasmin activity reflects more closely the extent of intraglomerular fibrinolysis while urinary plasminogen reflects non-renal proteinuria in patients with severe toxemia. In the normal healthy kidney only negligible fibrinolytic activity has been found (5). The slight increase in the levels of urinary plasmin during late pregnancy and the early puerperium in normal women are possibly related to the lysis of fibrin in the localized areas of the glomerular compartment (2). An enhanced fibrinolytic activity during labor may be explained by the damage during delivery and could be a natural defence mechanism which maintains the maternal blood supply to the placenta by ensuring the patency of the placental bed during labor (3).

The large amount of urinary plasmin in mild cases of toxemia suggests that active intraglomerular coagulation and fibrinolysis occurred and removed fibrin laid down in the glomeruli lead to a fibrinogen-dominant state in the urine.

In severe pre-eclampsia a marked diminution of urinary plasmin activity in the later part of the gestational period is considered to be an indirect but reliable sign of pronounced renal damage. The progressive decrease in the level of urinary plasmin indicated decompensated disseminated intraglomerular coagulation either due to an exhaustion of plasminogen activators or to an increased activity of the fibrinolytic inhibitors in the renal tract. Consequently a coagulation dominant picture was brought about in the glomeruli which represents a causative factor in the production of rapid deterioration of renal function during pregnancy. Following delivery a striking rise of urinary plasmin level together with rapid clinical improvement in Group A may indicate that a high fibrinolytic glomerular activity in toxemia during the puerperium is a such prognostically favorable sign that the complete evolution and resolution of the

glomerular lesion can be expected in this type of toxemia.

On the contrary the poor prognosis in severe toxemia of Group B seems to be due to a persistently low activity of postpartal urinary fibrinolysis which leaves intraglomerular lesions may result in residual proteinuria and hypertension in this type of toxemia.

The very high levels of urinary plasmin found in abruptio placentae may be important in understanding the pathogenesis of the defective hemostasis accompanying this complication and determination of plasmin in the urine could be of great value in the early diagnosis of this obstetric complication.

It is of great interest that extremely high levels of urinary plasminogen appeared while urinary fibrinolytic activity remained markedly depressed immediately after the convulsion of eclampsia in late pregnancy. However a pronounced increase in fibrinolytic activity during the first two weeks of the puerperium after an eclamptic attack is considered to be a natural healing process of impaired homostasis. Some authors have speculated that a toxic substance triggering the clotting mechanism in this disease stimulates aggregation of platelets to form thrombi which were swept into the microcirculation of the brain, liver and kidney damaging their vascular endothelium locally and producing alteration of the basement membrane permeability by which red blood cells were forced out into the perivascular region producing the ring hemorrhages found in all patients with eclampsia. Such hemorrhages with transient stasis of blood flow in the microcirculation are likely to result in typical acute intravascular coagulation in eclampsia (9, 14, 17).

The demonstration in this investigation of increased amounts of urinary plasmin suggests that intraglomerular fibrin deposition and fibrinolysis occur in varying degrees in normal parturition, toxemia of pregnancy, abruptio placentae and eclampsia.

The clinical outcomes of these complications in pregnancy probably depends on the balance between clotting and lysis as the condition evolves. Finally and perhaps most significantly of all in toxemia of pregnancy the serial estimation of urine plasmin activity may provide a tool which at minimal inconvenience to the patient may be of value in monitoring the progress of the disease and facilitate the continuous review of specific therapy (6, 18).

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Submitted for publication September 17 1979

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## FREEZE MICROSCOPY OF THE ENDOMETRIUM IN ECTOPIC PREGNANCY

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**Abstract** A series of 60 patients with suspected ectopic pregnancy was reviewed in order to assess the diagnostic value of freeze microscopy of endometrial curettages. By this procedure the correct diagnosis has been made in 100 per cent of patients i.e. it established whether the patient was pregnant and whether the pregnancy was intra- or extrauterine.

Eighteen patients had an ectopic pregnancy 13 of them were detected primarily on microscopy of frozen sections while the remaining 5 were erroneously classified as non-pregnant. The diagnostic sensitivity of freeze microscopy proved to be equal to microscopy of the subsequent paraffin sections.

A diagnostic and therapeutic schedule can be set up for patients with suspected ectopic pregnancy when the histological appearances of the frozen sections and the result of the HCG reaction are known.

The best known early gestational changes in the endometrium are the presence of decidua and the Arias-Stella phenomenon (11, 12) which is defined as the occurrence in endometrial glands of cells having an enlarged irregular hyperchromatic nucleus and pale vacuolated cytoplasm (1).

A somewhat less known morphological sign of pregnancy is the gestational hyperplasia described by Herzig in 1964 (6). This is the simultaneous presence in the endometrium of secretion, stromal edema and predecidua, three phases which occur sequentially in the non-pregnancy menstrual cycle.

The Arias-Stella phenomenon has been observed as early as the 22nd day after the first day of the last menstrual period (7) and gestational hyperplasia develops from the 23rd-28th day of the cycle. In other words these histological characteristics afford a possibility of a very early diagnosis of pregnancy including its ectopic variant.

In the Gynecological/Obstetrical Department of the Central Hospital Slagelse the ordinary clinical examination for ectopic pregnancy has been supplemented since 1971 by microscopic examination of frozen sections of endometrial material obtained

by curettage or vacuum curettage. However this method has only been used when the ordinary clinical examinations have not been able to prove or disprove a suspicion of ectopic pregnancy and the patient did not wish to continue a possible intrauterine pregnancy.

Accordingly the patients in the present series make up only a small part (about 20 per cent) of the total number admitted to the Department with a suspicion of ectopic pregnancy during the period under study. Patients having classical acute symptoms and signs of ectopic pregnancy were initially subjected to laparotomy or laparoscopy. Only patients giving rise to diagnostic difficulties and having metrorrhagia, menostasis and/or unilateral parametrial masses have had frozen section microscopy of the endometrium and were included in the present material.

## PRESENT INVESTIGATIONS

**Material.** The series is composed of 62 patients admitted during the period 25.8.71-29.11.77. However one patient entered the series twice and two were excluded: one because the curettages did not yield any tissue for a histological diagnosis and the other because the final clinical diagnosis remained uncertain (a regressed tubal abortion could neither be excluded nor confirmed). This leaves 60 patients in whom clinical examination initially gave rise to a suspicion of ectopic pregnancy without there being any indication for acute laparotomy or laparoscopy.

Table I gives the diagnoses on admission.

Table I Diagnoses on admission

	No of patients
Induced abortion	14
Threatening abortion, incomplete abortion or spontaneous abortion	14
Suspicion of ectopic pregnancy	24
Other diagnoses (metrorrhagia, ovarian cyst, acute appendicitis etc.)	8



Table II Relationship between frozen section microscopy and clinical diagnosis

	Clinical diagnosis		
	Ectopic pregnancy	Intrauterine pregnancy	Not pregnant
<b>Frozen section microscopy</b>			
Ectopic pregnancy	13	3	2
Intrauterine pregnancy	0	24	1
Not pregnant	5†	0	13
Total	18	27	15

† Re false negative frozen section microscopy of Table III

Re false positive frozen section microscopy of Table IV

**Method** In 30 cases the endometrial specimens were obtained by vacuum curettage using the Vabra aspirator<sup>2</sup> thus avoiding general anesthesia (unless in certain cases) and dilatation of the cervical canal. The remaining procedures were done by a conventional curette or else as a vacuum evacuation if the patient had been referred primarily for an induced abortion.

The material was sent immediately unfixed to the Department of Pathology where the tissue (as a rule *in toto*) was frozen cut into 8–10 µm sections in a cryostat and stained with toluidine blue/eosin as described by Henriques (5).

In all cases the histological diagnosis was obtained within 20 minutes. As detailed below it was designated positive if it was not intrauterine pregnancy (pregnancy product) or non pregnant. The subsequent procedure (laparoscopy, laparotomy or further observation) was decided on basis of the microscopic findings and clinical appearance.

Twenty nine patients had laparoscopy or laparotomy as a continued suspicion of ectopic pregnancy. All the others were followed until the clinical diagnosis had been fully established. Patients diagnosed as spontaneous

abortion or incomplete abortion<sup>1</sup> were designated as having intrauterine pregnancies if according to the clinical findings the abortion was assumed to have occurred not earlier than one month before the freeze microscopy.

## RESULTS

Among the 60 patients the correct diagnosis was made by frozen section microscopy in 49 (=82 per cent) (95 per cent confidence limits 69.6 per cent–90.5 per cent). The results are presented in Table 2.

In 18 patients the ectopic pregnancy was confirmed at operation. 13 of them had been diagnosed by frozen section microscopy (=72 per cent) (confidence limits 46.5 per cent–95.3 per cent). In the false negatives neither the frozen sections nor the final paraffin sections showed any signs of pregnancy.

In 5 patients who did not have an ectopic pregnancy the diagnosis after microscopy of the frozen sections had been 'possibly ectopic pregnancy'.

Tables 3 and 4 give the relationship between the histological findings on frozen sections, on paraffin sections and the final clinical diagnosis in the final reports.

## DISCUSSION

In previous studies on the diagnostic value of histological examination of endometrial tissue for possible ectopic pregnancy the diagnostic criterion for ectopic pregnancy has been mainly the occurrence of the Arias Stella phenomenon without the simultaneous presence of placental components or fetal loss.

Table III Relationship between frozen section microscopy and paraffin section microscopy in patients in whom the frozen sections had shown no pregnancy but in whom clinical examination revealed ectopic pregnancy i.e. the false negatives

No of patients	Diagnosis on frozen section microscopy	Diagnosis on paraffin section microscopy
7	Endometrium with necrotic tissue—placental remnants <sup>2</sup>	Endometrium in proliferative phase with signs of bleeding. Flakes of necrotic tissue.
15	Suspicion of dys-hormonal endometrium predominantly in secretory phase	Dys-hormonal endometrium of secretory type. No signs of pregnancy.
21	Endometrium without signs of pregnancy	Endometrium in uncharacteristic phase.
32	Endometrium in secretory phase with localized decidual stromal reaction	Endometrium in menstrual phase. No signs of pregnancy.
33	Endometrium in proliferative phase	Endometrium in proliferative phase. No evidence of pregnancy.

Table IV. Frozen section diagnosis, paraffin section diagnosis and final (clinical) diagnosis in patients who had been erroneously diagnosed on freeze microscopy as (possibly) ectopic pregnancy

No.	Frozen section diagnosis	Paraffin section diagnosis	Final (clinical) diagnosis
1	Decidual endometrium Ectopic pregnancy cannot be excluded	Villi demonstrable Pregnancy products	Intrauterine pregnancy
2	Secretory endometrium and ample decidua Ectopic pregnancy cannot be excluded	Decidua with occasional small particles of trophoblast	Bicornate uterus, curetted in non-pregnant horn. Laparoscopy
3	Endometrium with characteristics of pregnancy (no pregnancy products)	Dysfunctional endometrium with localized decidual endometrium (seq. to pregnancy)	Probably non-pregnant, induced abort on 4 weeks previously Operation
4	Endometrium with decidua and Arias-Stella phenomenon	Endometrium with characteristics of pregnancy (no pregnancy products)	Spontaneous abortion 2. hours
5	Mild Arias-Stella phenomenon and secretory decidual reaction Possibly pregnant	Dysfunctional endometrium Possibly pregnant	Metrorrhagia. Not pregnant Operation

This criterion has been fulfilled in about 70 per cent of the patients with confirmed ectopic pregnancy. In practically all studies (2, 3, 4, 8, 9, 10) in the remaining 30 per cent it has not been possible to find definite signs of pregnancy in the endometrial tissue, presumably because of the death or insufficiency of gestational tissue in respect of hormone production prior to the time of curettage. Owing to this large number of false negatives microscopic examination of the endometrium on suspicion of ectopic pregnancy has rarely been used. Its applicability has been further restricted by having to wait 24–28 hours—without the conventional histological technique—for the microscopic diagnosis.

The use of freeze microscopy does not appear to have been reported previously in this connection. In the Department of Gynecology/Obstetrics in the Central Hospital Slagelse frozen section microscopy of the endometrium has proved to be a rapid, reasonably easy and valuable method. This applies particularly when the material has been obtained by the Vabra aspirator<sup>R</sup> and without the need for anesthesia. It is remarkable that the frozen section method has proved to be as sensitive as the conventional method using paraffin sections. Like others before us we must admit that about 30 per cent of the ectopic pregnancies were not recognized by microscopic examination of the endometrium, presumably owing to insufficient secretion from the ectopic gestational site and a resulting lack of endometrial reaction, viz. the Arias-Stella phenomenon and/or gestational hyperplasia.

Should the use of frozen section microscopy of the endometrium come into wider use in cases where the

diagnosis of ectopic pregnancy is in doubt, we feel that we can recommend the following guidelines.

(1) If microscopic examination of frozen sections reveals intrauterine pregnancy, the suspicion of ectopic pregnancy must be considered to have been disproved. The diagnosis of intrauterine pregnancy is based upon the demonstration of placental components and/or fetal tissue in the curettages which is not difficult in frozen sections. (Indeed our series does not include any patients with ectopic pregnancy who have been diagnosed as having intrauterine pregnancy.) Such patients (who made up 42 per cent of our series) can be immediately discharged for continued out-patient follow-up, unless this is contra-indicated by other diseases. This is an essential advantage, especially when the suspicion of ectopic pregnancy has arisen in connection with an out-patient procedure for induced abortion.

(2) If the frozen section microscopy gives rise to a suspicion of ectopic pregnancy (i.e. shows the Arias-Stella phenomenon and/or gestational hyperplasia without the presence of fetal or placental components) this is an indication for immediate laparoscopy or laparotomy in 30 per cent of our patients. In our series 72 per cent of these patients proved to have ectopic pregnancy.

(3) This leaves 17 patients (28 per cent) in whom frozen section microscopy showed no signs of pregnancy. Three of these cases had a positive urinary HCG reaction. If the HCG is positive laparoscopy should be carried out as the microscopic report no signs of pregnancy practically rules out an intrauterine pregnancy. Ectopic pregnancy was found in two of our three patients in this group. The

patient had a positive HCG reaction on admission but the reaction changed to negative during her stay in hospital. Thirty four days before admission the patient had undergone induced abortion (which showed fetal components) and the post abortion course had been complicated by long lasting metrorrhagia. On the basis of the positive HCG reaction laparoscopy was performed but did not show any signs of an ectopic pregnancy.

In our opinion patients with a frozen section microscopic report of no signs of pregnancy and a negative HCG reaction but with a continued clinical suspicion of ectopic pregnancy should be kept in hospital and observed. In our series this applied to 14 patients (23 per cent of the whole series) 3 (21 per cent) of whom proved to have ectopic pregnancy. In such cases the further procedure must be based upon the clinical state.

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Submitted for publication December 7 1978

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## HYDROP DEGENERATION

A histopathological investigation of 260 early abortions

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Placental tissue from 160 abortions provoked during the 6th-12th weeks and 100 spontaneous abortions between weeks 5 and 16 were studied histopathologically in order to make a comparison and quantitation of hydrop degeneration.

Significantly more cases of severe hydrop degeneration were observed among the spontaneous abortions ( $p < 0.001$ ). Of these abortions 87 per cent were hydrop degenerated to varying degrees, 41 per cent were severely hydrop degenerated. Among the provoked abortions approximately 51 per cent were hydrop degenerated to varying degrees and only 6.9 per cent were severely hydrop degenerated. The relationship between severe hydrop degeneration and chromosome abnormality is discussed.

Hydrop degeneration of the chorion villi is a common histopathological finding in placentae from early (1st trimester) abortions and it is a normal finding in a small area between the chorion leave and chorion trophoblast (5, 8). In addition a strong correlation has been demonstrated between chromosomal abnormalities in the aborted tissue and hydrop degeneration (2, 9, 12, 13, 18).

A considerable variation in the severity of placental hydrop degeneration has been observed during routine examination of tissue from both early spontaneous abortions as well as early provoked abortions. This routine examination has revealed placentae with only a few hydrop-degenerated villi as well as placentae in which an overwhelming majority and in a few cases all the villi were hydrop degenerated. Despite this the authors shown in Table I have not quantitated their findings of hydrop degeneration. This has given rise to considerable variation in the incidences quoted. The object of the present investigation had been partly to quantitate hydrop degeneration in the chorion villi from early placentae and partly to compare the findings from spontaneous and provoked abortions.

## MATERIAL AND METHODS

The material comprises placental tissue from 100 early spontaneous abortions and from 160 abortions provoked during the 1st trimester. The investigation is consecutive except that preparations with less than 800 chorion villi were excluded.

All the abortions took place in the latter half of 1976 in the Obstetric Department of Odense University Hospital.

No prostaglandin or saline induced abortions are included in the material.

The average gestational age of the spontaneous abortion was 11 weeks (range 5-16 weeks) and of the provoked abortions 9 weeks (range 6-12 weeks).

The age distribution of the women is shown in Fig 1. The average age of those with spontaneous abortion was 27.6 years and of those with provoked abortion 27.2 years. None of the women occur more than once in the material.

The placental material was fixed for at least 24 hours in 10 per cent buffered formalin whereafter 2-6 capsules were filled with tissue for embedding in paraffin. Thereafter

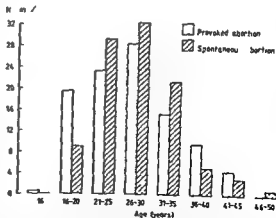


Fig 1 The number of women in the various age groups. Note that approximately 50 per cent of the provoked abortions were carried out on women in the age group 20-30 years and that approximately 60 per cent of the spontaneous abortions occurred within this age group. Only one of the 160 women subjected to provoked abortion was below the age of 16 years.



*Fig 2* Hydrop degeneration of grade I. Only a few of the chorion villi have vacuolized and edematous mesoblast (one arrow) and narrow single layered trophoblast (two arrows). Further on the absence of vessels in the villi. Normal chorion villi (three arrows) with loose stroma and double layered trophoblast ( $\times 100$ )

the tissue was cut into  $6\ \mu$  thick sections and routinely stained with hematoxyline eosin for light microscopic examination. Typical hydrop-degenerated chorion villi have edematous vacuolated mesoblast. The blood vessels are small or completely absent. The structures of the chorion villi are often smoothed with a narrow single layered trophoblast (see Fig 3).

The quantitation of the hydrop degeneration was carried out as follows:

*Grade 0* Less than 1 per cent hydrop-degenerated chorion villi per placenta

*Grade I* 1–20 per cent hydrop-degenerated chorion villi per placenta

*Grade II* 21–40 per cent hydrop-degenerated chorion villi per placenta

*Grade III* More than 40 per cent hydrop-degenerated chorion villi per placenta

## RESULTS

The distribution of the hydrop degeneration in respect of severity corresponding to provoked and spontaneous abortions is shown in Table 2. It may be seen that only 18.6 and 13 per cent of the examined

*Fig 3* Hydrop degeneration of grade III. Large edematous and vacuolated chorion villi which in several places are covered with single layered trophoblast (one arrow). The majority of the chorion villi have no vessels though a villus with hypoplastic vessels can be seen (two arrows) ( $\times 150$ )



Table I Some earlier studies on the incidence of hydrop degeneration in spontaneous abortions

Author and Year	Incidence in per cent
Gray (1957)	2.7
Dobrowie and Pisarski (1967)	10
Nayak (1968)	14
Jvirt (1957)	18
Elkmann and Carrow (1962)	29
Mall and Meyer (1921)	31.5
Huber Mellin and Vellios (1957)	40
Abasi and Aterman (1968)	41.3
Elke, Bailo and Finzi (1969)	52.5
Upadhyay, Prakash and Singh (1967)	60
Berg and Edmonds (1940)	66.9

among pathological ova

placentae respectively were found to have less than 1 per cent hydrop-degenerated chorion villi. Forty-one per cent of the placentae from the spontaneous abortions had severe hydrop degeneration compared with only 6.9 per cent of those from the provoked abortions ( $p < 0.001$ ). The hydrop-degenerated chorion villi either lay in small clusters or were spread diffusely throughout the placental tissue (Fig 2, grade I; Fig 3, grade III).

No qualitative difference was observed between the hydrop degeneration in the spontaneous and provoked abortions. In the great majority of cases the hydrop-degenerated villi were without blood vessels or the blood vessels were hypoplastic.

No abnormal trophoblast hyperplasia was observed. On the contrary the trophoblast layer appeared thinner than usual and often consisted of only a single row of cells in the swollen and edematous villi (Fig 3).

## DISCUSSION

It can be seen from Table 2 that hydrop degeneration of varying degrees is present in 87 per cent of the placentae examined from spontaneous abortions and in 81.4 per cent of those from provoked abortions.

These figures are somewhat higher than those reported previously (Table 1). However the studies referred to in the Table give no quantitation of the hydrop degeneration and it is therefore extremely difficult to make any comparison either between the incidences stated in the various publications or between these and the present findings. The reason for this is that much will depend on the individual author's ideas as to how many chorion villi must be hydrop degenerated in a placenta before this is classified as hydrop degenerated.

In addition to these purely quantitative problems there are a number of qualitative questions which are still unsolved. Thus some authors distinguish between hydrop degeneration without trophoblast hyperplasia and hydatidiform degeneration with trophoblast hyperplasia (20). No abnormal trophoblast hyperplasia was observed in the present study; neither was there any case with clinical or histological evidence of a hydatidiform mole. Further, other authors only make the diagnosis hydrop degeneration if macroscopic examination with the naked eye of the aborted tissue discloses vacuolated chorion villi. In addition to these problems of definition there were difficulties in earlier investigations in determining whether an abortion was in actual fact spontaneous or criminal (17). It has been possible to ignore this problem in Denmark because free abortion became a legal right as of October 1973, so that the number of criminal abortions since then is no doubt extremely low.

It would be desirable to have both a quantitative and a qualitative standardization of the criteria for hydrop degeneration, as in particular the studies of the last 10–15 years of immature placentae have shown a strong correlation between hydrop degeneration and abnormal chromosomes in abortion tissue. Thus Honore *et al.* using light microscopic evaluation of placentae have been able to predict chromosome abnormality with considerable accuracy.

It can be seen from Table 2 that the number of hydrop-degenerated chorion villi is much the same in

Table II The incidence of hydrop degeneration of various degrees of severity in placental tissue from provoked and spontaneous abortions. The figures in brackets are percentages

	Grade (hydrop degeneration)				Total
	0	I	II	III	
Provoked abortions	30 (18.6)	97 (57.5)	77 (16.9)	11 (6.9)	160 (100)
Spontaneous abortions	13 (13.0)	37 (37.0)	9 (9.0)	41 (41.0)	100 (100)
Total	43 (16.5)	134 (49.6)	86 (33.8)	52 (20.0)	260 (100)

spontaneous and provoked abortions until approximately 40 per cent of the placenta is involved. It is only after this stage (grade III) that a sharp rise occurs in the number of hydrop-degenerated placentae in spontaneous abortions (chromosome abnormality?). Thus hydrop degeneration of grade III is found significantly more frequently among spontaneous abortions than among provoked. It can further be seen from Table II that a lower degree of hydrop degeneration (grades I and II) is exceedingly common and therefore in all probability a harmless phenomenon in early placentae from both spontaneous and provoked abortions.

An investigation regarding a possible correlation between chromosome abnormalities and the histopathology of abortion tissue is under preparation in our laboratory.

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Submitted for publication September 17 1979

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## INFERTILITY FACTORS

Their relative importance and share in an unselected material of infertility patients

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**Abstract** A 7 year material of 196 infertile couples is presented 1.5-8.5 years after investigation and/or treatment of infertility rather more than 48 per cent of the women had conceived and of these pregnancies 19 per cent ended in abortion.

Various factors such as duration of infertility age history of pelvic inflammatory disease and previous use of oral contraceptives are analyzed for their relative share in the material and relative importance for the chances of pregnancy.

—Unlike age the duration of infertility was an important prognostic factor also when it exceeded 2 years previous operations on the internal genitalia reduced the chances of conceiving essentially more than a history of one episode of pelvic inflammation whereas several episodes of pelvic inflammation seemed to spell an equally unfavorable prognosis. As expected infertility caused by oral contraceptive-conditioned oligo-amenorrhea was considerably easier to treat than other forms of infertility. Oligo-amenorrhea was significantly more common among patients who had used oral contraception for 1-6 years than among those who had used it for less than 1 year. —The incidence of abortion in patients with secondary infertility was about three times higher before than after the infertility investigation.

Lastly the individual infertility diagnoses were analyzed the same way as regards relative share and importance about 42 per cent of the operated patients succeeded in conceiving. It is remarkable that 8 patients had a bicornuate uterus and of them 7 had oligo- and/or hypomenorrhea.

Several factors are involved in human reproductive ability. The percentage share of unknown factors among infertile couples has ranged from 5-20 per cent (4, 6). In materials in which an attempt has been made to assess the frequency of a multifactorial etiology up to 45 per cent (3) have proved to belong to this category. Therefore it may be difficult in many respects to set up applicable prospective and controlled studies to elucidate the influence of individual factors upon infertility. The object of the present study was to analyze an unselected material of infertile couples investigated and treated for the infertility especially with reference to the fertility reducing effect of the individual factors.

## MATERIAL AND METHODS

During the 7 year period from 1.1.1967 to 31.12.1973 a total of 196 women including 4 previously sterilized were admitted to the Gynecologic Department of the Central Hospital Naestved for investigation and treatment of infertility. Only 14 (7.1 per cent) did not want further investigations after the first consultation. The analysis was done by collecting data from the case records and from questionnaires. Twenty five patients (12.8 per cent) could not be traced. The analysis was terminated in the autumn of 1975 so that the follow up period ranged from 1.5 to 8.5 years.

Fig. 1 sets out the distribution by age and by primary and secondary infertility. 56.8 per cent applied because of primary and 43.2 per cent because of secondary infertility. 77.6 per cent of the patients were under 30 years of age when first seen. Eleven patients (5.5 per cent) were over 35 years and of this group 6 were applying because of primary infertility. In almost one-quarter of the patients the duration of infertility was 1-2 years in almost one half from 2-4 years and in almost one third more than 5 years.

The majority of the patients a total of 128 were admitted during the initial diagnostic work up and thereafter attended as out patients for continued investigations and/or treatment. Twenty nine patients had previously been investigated and treated for infertility.

As a routine general clinical and gynecological examinations were performed. The anatomical condition was assessed by hysterosalpingography (HSG). In 16 cases HSG was not performed because in 10 patients the wish to conceive was not so ardent or because the patient refused this examination. Two conceived before the radiography was done and in four HSG was omitted because the patients had previously been sterilized.

The presence of ovulation was assessed primarily by basal temperature curves through 2 or 3 months. In the meantime some of the patients conceived. Measurement of total estrogens and pregnandiol in the 24-hour urine and endometrial biopsy were done in rather more than half the patients. Other investigations for endocrine status including determination of pituitary gonadotropin, adrenocortical and thyroid function parameters were carried out when this was felt to be indicated.

No attempt was made to assess the role of the cervix factor in infertility as detailed studies of this factor were not initiated until the early 1970's. However a few patients in whom cervicitis was the only abnormal finding were assigned to this group.

Investigations of semen were carried out in the laboratory



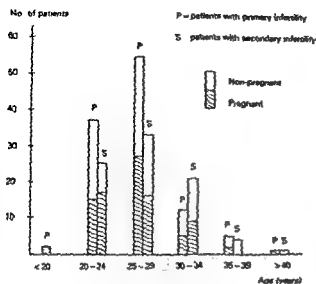


Fig. 1 Conception rate by age for patients with primary and secondary infertility respectively

of the Central Hospital. Thirty five refused to have this investigation or postponed it so that several of the women had conceived in the meantime. In 13 cases the reason why the husband's sperm was not studied is not stated. In cases of male infertility the husband was referred to an andrologist for special treatment.

## RESULTS

Of the total material of 196 patients 94 or 47.7 per cent conceived. Eighteen or 19.1 per cent aborted, assuming that the 25 patients who could not be traced had the same conception rate as those who could be followed. The conception rate in the total series is 54.6 per cent. Below the rate will be given mostly without this correction and is thus a minimum value. When the rate is followed by a figure in brackets the correction has been made. Of the 111 patients with primary infertility 52 or 46.8 per cent conceived and of the 85 with secondary infertility 42 or 49.4 per cent. The difference is not statistically significant (chi square test  $p > 0.05$ ). The duration of infertility

Table I Influence of duration of infertility upon conception rate

Duration of infertility	No. of patients	No. and percentage who conceived
1-2 years	44	33 (75.0)
2-4 years	108	52 (48.2)
>4	44	9 (20.5)

the figures within parentheses denote numbers in per cent

Table II Abortion rate for patients with secondary infertility before and after investigation and/or treatment for the infertility

	Before investigations for infertility	After investigations for infertility
No. of pregnancies	112	41
Abortions	32 (28.6)	5 (12.2)

the figures within parentheses denote numbers in per cent

seemed to be longer for the patients with primary infertility than for the secondary cases: a duration of less than 4 years being found in 71 = 64 per cent and 64 = 75 per cent respectively. However, this difference is not significant (chi square test  $p > 0.05$ ).

The duration of infertility was correlated to the conception rate in Table I. It is apparent that the conception rate falls with increasing duration of infertility. The differences are statistically significant between all three groups (chi square test  $p < 0.01$ ). The mean duration of infertility for those who conceived and for those who did not was calculated as 2.1/4 and 4 years respectively. The respective 95 per cent confidence limits are then 2.0-2.5 years and 3.5-4.5 years (SEM<sub>1</sub> 0.17 and SEM<sub>2</sub> 0.25).

The influence of age upon the chances of pregnancy may be seen from Fig. 1. Analysis of the differences between the 5 year groups showed no statistical significance.

In the analysis of the collected anamnestic data, particular interest was taken in a history of abortion of operations on the internal genitalia, of pelvic inflammatory disease and of the use of oral contraceptives.

A history of spontaneous abortion in patients with secondary infertility was elicited in 32 out of a total 112 pregnancies = 28.6 per cent (95 per cent confidence limits 20.9-38.6 per cent). After the investigations and treatment for infertility the same patients obtained 41 pregnancies = 12.2 per cent of which terminated in abortion. (The calculations do not include patients who had previously been sterilized.) This difference in the abortion rate before and after the investigations for infertility is significant (chi square test  $p < 0.05$ ) cf. Table II. — The duration of infertility and the time of conception after the investigations had been completed was compared for patients with and without a history of abortion (Table III) with a view to possibly demonstrating a lower fertility in the former group. No such finding is

Table III Duration of infertility and time of conception after investigation and/or treatment of patients with a history of abortion and patients who had no such history

	Duration of infertility (years)			Time of conception (years)		
	≤2	>2<4	≥4	≤2	>2<4	≥4
Patients with a history of abortion (3) <sup>†</sup>	9 (28.1)	14 (43.8)	8 (25.0)	14 (77.8)	2 (11.1)	2 (11.1)
Patients with no history of abortion (164)	35 (1.3)	76 (46.3)	53 (31.3)	60 (18.9)	13 (17.1)	3 (3.9)

the figures within parentheses denote numbers in per cent

† the figures within parentheses denote number of patients

apparent from the table but it shows a shorter duration of infertility among patients with a history of abortion.

A history of *extrauterine pregnancy* was reported by 5 patients = 5.9 per cent. In all of them one Fallopian tube had been excised. Three of the patients with a normal remaining tube at HSG conceived and delivered. The other two had sacosalpinx and did not conceive.

Fifteen patients had a history of *operation on the internal genitalia* (this does not include the four who had undergone sterilization). At HSG 7 of these patients or 46.7 per cent had obstruction to passage in both tubes and 5 or 33.3 per cent succeeded in conceiving. Within the remaining part of the material bilateral tubal occlusion was found in 38 cases = 20.3 per cent. The differences in the occurrence of bilateral tubal occlusions and in the conception rates between the group with a history of operation on the internal genitalia and the remainder of the material are statistically significant (chi square test  $p < 0.05$ ).

Among other anamnestic data which may be of causal importance to infertility there is a history of *pelvic inflammatory disease*. Such a history was elicited in 57 or 29.1 per cent, one episode in 35 of them and two or more episodes in 22. Twenty-eight or 48.3 per cent achieved pregnancy which does not

indicate a poorer prognosis for patients with a history of pelvic inflammation than for the remainder of the material. From Table IV it is apparent that patients with a history of one episode of pelvic inflammation had the same chances of conceiving as the other infertility patients. On the other hand repeated pelvic inflammations impair the chances of pregnancy but only in the presence of tubal occlusion on the HSG. However the difference in the conception rate between these two sub-groups is not statistically significant (Fisher's exact test  $p > 0.05$ ). In more than half of both sub-groups or more accurately in 20 and 12 patients with a history of one and of several episodes of pelvic inflammation the tubes were normal at HSG and in these patients the conception rate was the same as in the other infertility patients.

Seventy-six patients or 38.8 per cent of the total material had used *oral contraceptives* for up to 6 years, 40 of them for less than one year, 25 for 1–2 years, 10 for 3–4 years and one patient for 5–6 years. — To assess the importance of previous oral contraception as a cause of infertility the use or non-use of oral contraceptives is correlated in Table V with major HSG findings with bleeding disturbances at the time of the first visit and with the conception rate. A comparison of patients who had not used oral contraception with patients who had used the Pill for a short time and patients who had used it for a long time shows in this sequence a fall in the frequency of abnormal HSG with tubal occlusions and an increasing frequency of bleeding disturbances in the form of oligo- and amenorrhea at the first consultation. The differences in the frequency of tubal occlusions and the occurrence of oligo- and amenorrhea between users and non-users of contraceptive pills are statistically significant. So is the difference in the frequency of oligo- and amenorrhea between patients who had used oral contraception for less than one year and patients who had used it for 1–6 years (chi square test  $p < 0.05$ ). From the same table it

Table IV Presence of tubal occlusions and conception rate in infertility patients having a history of one or more episodes of pelvic inflammatory disease

No. of episodes of pelvic inflammatory disease	Tubal occlusions		In all	Conceived
	Partial	Total		
One episode (35) <sup>†</sup>	7	8	15 (42.8)	7 (46.7)
Two or more (22) <sup>†</sup>	4	6	10 (45.5)	1 (10.0)

the figures within parentheses denote number in per cent

† the figures within parentheses denote number of patients

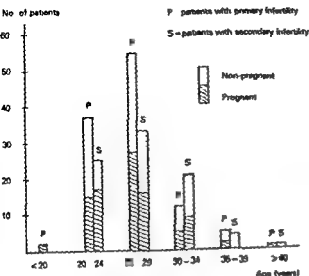


Fig 1 Conception rate by age for patients with primary and secondary infertility respectively

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## RESULTS

Of the total material of 196 patients 94 or 47.7 per cent conceived. Eighteen or 9.1 per cent aborted. Assuming that the 25 patients who could not be traced had the same conception rate as those who could be followed the conception rate in the total series is 54.6 per cent. Below the rate will be given mostly without this correction and is thus a minimum value. When the rate is followed by a figure in brackets the correction has been made. Of the 111 patients with primary infertility 52 or 46.8 per cent conceived and of the 85 with secondary infertility 42 or 49.4 per cent. The difference is not statistically significant (chi square test  $p > 0.05$ ). The duration of infertility

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In the analysis of the collected anamnestic particular interest was taken in a history of abortion of operations on the internal genitalia of pelvic inflammatory disease and of the use of oral contraceptives.

A history of spontaneous abortion in patients with secondary infertility was elicited in 32 out of a total of 112 pregnancies = 28.6 per cent (95 per cent confidence limits 20.9-38.6 per cent). After the investigations and treatment for infertility the same patients obtained 41 pregnancies = 12.2 per cent of which terminated in abortion. (The calculations do not include patients who had previously been sterilized. This difference in the abortion rate before and after the investigations for infertility is significant (chi square test  $p < 0.05$ ) cf Table II. — The duration of infertility and the time of conception after the investigations had been completed was compared for patients with and without a history of abortion (Table III) with a view to possibly demonstrating a lower fertility in the former group. No such finding

Table VII Types of surgery

Type of surgery	No. of patients	No. who conceived	No. of years after surgery			
			0-1	1-2	2-4	>4
Bilateral salpingostomy	17	3	1		2	
Unilateral salpingostomy	5	2	2			
Unilateral salpingectomy	5	2				2
Replantation of tubes (after sterilization)	4	1				1
Detachment of adhesions	4	2	2			
Ovarian resection	4	4	2	2		
Mionectomy	3	2		1		1
Metrosuspension of the uterus	9	5	3	2		
Strassmann's operation	1	1	1			
Total	52	22	11	5	2	4

the first year after the treatment. Another 12 patients were treated with Clomivid<sup>®</sup> because the basal temperature curves indicated questionable ovulation. On this treatment 90 per cent exhibited definite ovulation and 80 per cent conceived, the majority within the first year after the treatment.

Abnormalities of the semen as the only cause of infertility were demonstrated in only 19, or 9.7 per cent of the cases. Pregnancy was obtained in 8 = 42.1 per cent. The small number in which male infertility was the only cause is explicable partly by the large number of husbands who refused to have the analysis. Among the 36 cases of unknown cause of infertility 10 of the husbands had not been examined. Further investigation of these 10 patients' data showed that in the whole their motivation for the investigations was slight.

In 26 patients, or 13.3 per cent, the diagnostic programme revealed multiple diagnoses. This number could presumably have been larger if a more consistent study had been made of the role of the cervix factor and if laparoscopy had been done to disclose en-

dometriosis and adhesions around the internal genitalia. Such changes are frequently not detected at HSG. Information about the husband's semen was lacking for 5 patients in this group.

It is remarkable that in 7 patients with oligo- and/or hypomenorrhea HSG showed a slightly bicornuate or a unicornuate uterus. None of the patients without oligo- or hypomenorrhea had any form of uterine developmental anomaly except for one who underwent a Strassmann operation. In this patient the bleeding pattern was completely normal. Within the total series 65 patients had miscellaneous bleeding disturbances when first seen. This includes 38 with oligo- or amenorrhea and 3 with hypomenorrhea. There was a statistically significant difference in the occurrence of bicornuate and unicornuate uterus among these types of bleeding disturbances compared with the remainder of the infertility patients (chi square test  $p < 0.01$ ). Table VIII gives the bleeding intervals and the previous use of oral contraceptives for the above mentioned 7 patients. Hormone analyses including thyroid parameters were normal in all

Table VIII Data for patients with bicornuate uterus and oligo- or hypomenorrhea

Age (years)	History of previous delivery	History of previous abortion	Had used oral contraceptives	Intervals between menstrual periods	Hypomenorrhea	+/- ovulation
3	+		1-2 months	4-8 weeks	+	+
4	+		12	8-24		-
5	+	+	-	2-6		+
5			6	4-8		+
6			24	4-11		+
20			24	4-8	+	+
32	+	+	6-8	4	+	+

7 In one patient however the hormone analyses were not complete — In 5 of the 6 patients who had previously used oral contraceptives the bleeding disturbances had started before she began using this form of contraception

Out of the total of 94 patients who conceived after investigation and/or treatment 55 or 58.5 per cent had conceived within one year 19 or 20.2 per cent within 2 years 15 or 16.0 per cent within 4 years and 5 or 5.3 per cent later Among the 102 patients who did not conceive 52 stated at the time of follow up that they no longer wanted to become pregnant Eighteen reported having adopted children

## DISCUSSION

The present infertility material does not differ essentially from most others with respect to age primary and secondary infertility percentage completing the diagnostic work up and tracing percentage (1 4 6 8 9 11) In most studies as here the infertility limit has been set at 1 year's involuntary childlessness partly because in almost 90 per cent of fertile couples conception occurs within 12 months (16) partly because it cannot be expected that the chances of conception increase with time in certain cases of absolute infertility On the other hand differences in the length of the follow up period in the various analyses of infertility materials can essentially influence the values for the prognostic results, but cannot reduce the comparability as regards the relative share of the individual infertility factors and the role of fertility suppression The present patients must also be presumed to have been largely unselected as there was no primary gynecological service in the area at the time However it may be imagined that in a few cases in which the patient's own doctor has found aspermia or considerably reduced fertility in the husband the case has been referred directly to an infertility clinic with andrological sub speciality

There was no definite difference in the conception rate between primarily and secondarily infertile patients Yet there has been a general trend to a somewhat better prognosis for secondary infertility in the various studies although in most material the differences have not been significant (1 3 9) Only Southam & Buxton (11) found a significant difference This may indicate a real difference in fertility between the two groups but the explanation could also be that the secondarily infertile patients have a

shorter duration of infertility presenting themselves earlier partly because of an older mean age and partly because of a possible tendency to abortions 1 De George & Nesbitt (3) we found a shorter duration of infertility among the patients with secor in those with primary infertility In general this is tantamount to a higher conception rate as is apparent from Table I

The marked fall in the conception rate when duration of infertility increases from 1 to 2 years or more agrees with previous findings (1 6 9 11) Also a further sub-division of durations of infertility past 2 years often shows a continued decrease in the conception rate However there do not seem to be other studies demonstrating—as here—a significant difference between the groups whose infertility exceeds 2 years (Table I) This *per se* renders the duration of infertility an important factor in the reproduction potential As a result it is more difficult to doubt the infertility problem in patients with less than 2 years infertility as Southam & Buxton (11) do —The significant difference in the median duration of infertility between patients who achieve pregnancy and those who do not agrees with the findings of De George & Nesbitt (3)

The effect of age on infertility has been investigated just as often as the influence of the duration of infertility Several authors have found a decreasing fertility, in terms of achieved pregnancies with increasing age (1 6 8 11) Studies in which an attempt has been made to correct for the partial incorporation of age in the duration of infertility have shown like ours no influence of age upon the chance of conception (3 4)

A 2 to 4 times higher abortion rate among with low fertility or infertility compared with normally fertile women has been substantiated only a few times previously in similar materials Southam (11) found in accordance with the present study a decrease in the tendency to abortion after investigation and treatment for the infertility When the fertility increases the abortion rate decreases However the fertility in patients with a history of abortions does not increase to the extent of being reflected in the time of conception after investigation and treatment for the infertility in relation to other infertility patients (Table III)

It has previously been demonstrated that a history of operations on the internal genitalia is tantamount to decreased chances of conception (7) The prognosis for these patients is essentially poorer than for

other infertility patients. Between the two groups there is a significant difference in the occurrence of bilateral tubal occlusions which *ceteris paribus* is an unfavorable infertility diagnosis as regards treatment.

About 30 per cent of the present patients had a history of pelvic inflammatory disease once or several times. According to our study this group as a whole does not have a poorer prognosis than the other patients. It was only in cases with a history of several episodes of pelvic inflammation and at the same time tubal occlusions at HSG that the chances of pregnancy were reduced. Lamb (7) who made no distinction between patients with one or more episodes of pelvic inflammation found a tendency to poorer chances of pregnancy in the total group but was unable to draw definite conclusions because the material was too small.

To assess the role of oral contraception in an ordinary unselected infertility material the frequency of two accepted infertility factors — one of which may be pill-dependent and the other hardly so — were compared for women who had and had not used oral contraceptives. The results must be interpreted as a sign that oral contraception suppresses fertility by the development of oligo- and amenorrhea. The chronological relation between discontinuing the Pill and the development of infertility is not stated. For most patients however the oral contraception was discontinued because a pregnancy was desired but in several cases this wish did not arise until a varying period had elapsed after the discontinuation. However the oral contraceptives may still be a cause of infertility as demonstrated by Vessey *et al* (13). These authors found a decreasing fertility suppressing effect of oral contraceptives over a period of 30–42 months after discontinuation. It is not apparent from their publication whether the infertility was due to oral contraception induced oligo-amenorrhea. — If a causal relationship did exist between the use of oral contraception and the development of oligo-amenorrhea induced infertility in all the 23 patients in our material who had these bleeding disturbances the remaining patients who had used oral contraception but without developing oligo-amenorrhea (76–23=53) would be expected to show just the same frequency of tubal occlusion at HSG as patients who had not previously used oral contraception (i.e. 40 per cent). But instead of 40 per cent of 53=22 that might be expected there were only 15 (Table V). This difference is not statistically significant but nevertheless great and it indicates that oral

contraception may give rise to infertility problems even though the patient does not develop oligo- or amenorrhea.

The distribution of the individual infertility diagnoses in the material accords fairly well with previous findings in similar materials. The reason for the small number with exclusively male infertility has been discussed above. The surgical results seem to be as satisfactory as in the materials of Swolin (12) and Jessen (5). Of our 52 operated patients 22 or 42.3 per cent (48.9 per cent) achieved intrauterine pregnancies compared with 38 per cent of 52 patients in Jessen's material and 22 per cent of 40 in Swolin's. Among patients who underwent bilateral salpingostomy 3 out of 17=17.6 per cent conceived compared with 36 per cent of 25 in Jessen's material and 18.2 per cent of 33 in Swolin's. The comparability of series undergoing operation for infertility even when stating all definable factors that are likely to affect the result can hardly ever be satisfactory because of the innumerable arbitrary factors in the anatomical pathogenesis of infertility.

The individual infertility diagnoses will not be discussed in detail. Suffice it to mention that the uterine infertility factors are in agreement with those normally accepted except for the group of uterine retroflexion which is generally not considered a cause of infertility (14). A recent study (15) has shown among 1954 pregnant women a statistically significant difference in the frequency of bleeding and abortion in the first trimester between women with and without retroflexion of the uterus. This might indicate a lower fertility in women with retroflexion of the uterus.

The number of infertility patients with a multifactorial etiology must be expected to increase when modern diagnostic methods are applied as already discussed. Whether a causal relationship does exist between the uterine developmental anomalies diagnosed at HSG and mild bleeding disturbances — such as oligo- and hypomenorrhea — cannot be established for certain in spite of the convincing findings as these findings were accidental and not primarily included in the aim of the study. However the theory of such a relationship is supported by another study admittedly using the same methodology. Semmens (10) found mild oligomenorrhea in 8 per cent of 25 patients with uterus bicornis unicollis, uterus septus, uterus subseptus and uterus arcuatus. This bleeding disturbance is not further commented in his publication and it does not appear to occur in

patients having other congenital anomalies or in 220 out of 500 pregnant patients with developmental anomalies collected from the literature on uterine developmental anomalies in which the menstrual cycle is described. On the other hand menorrhagia and dysmenorrhea are common. Only a few of Semmens' own patients had presented themselves because of infertility problems so that his material differs from ours in this respect. Except for uterus septus all the other three uterine anomalies in Semmens' study show the same appearances on the HSG (2). Five of the 7 patients in our material with oligo- and/or hypomenorrhea exhibited such uterine cavities. In one the HSG appearance suggested a uterus septus or bicornis bicolis and in one a uterus unicornis.

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Submitted for publication February 21, 1979

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# GONORRHEAL INFECTION FOLLOWED BY AN INCREASED FREQUENCY OF CERVICAL CARCINOMA

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**Abstract** In a town with a fairly centralized health service and a well organized gynecologic health control women who had had gonorrhea in 1954 or 1955 were reviewed for presence of cervical neoplasia and compared with age matched controls. Of 164 women studied 29 (17.7 per cent) had 4 or 23 years later developed cancer *in situ* compared with 1 (4.3 per cent) of the controls ( $p < 0.001$ ) while 8 (4.9 per cent) had invasive cancer *in situ* against only one (0.6 per cent) of the controls ( $p < 0.02$ ). Malignant disease of the cervix proved at least four times as common among the women with gonorrhea in their history as among the controls. The findings corroborate the view that cervical carcinoma is a sexually transmitted disease.

It may be assumed that at least every fourth woman who had gonorrhea had been or is a carrier of the carcinogenic agent(s). It is probably easier to search for and detect such agents in association with gonorrhea than in patients with already manifest cancer of the uterine cervix.

Sexual intercourse is thought to play an important role in the development of cervical carcinoma. The disease is also more common among married women (5). It is rarely diagnosed in nuns (3). Its frequency is increased among women who had their first pregnancy early in life (8) and among women who started their sexual activity while still relatively young (16). Sexual promiscuity (15), prostitution (13) and low socio-economic status (6) are other factors related to increased frequency of carcinoma of the cervix. Further evidence of sexual intercourse being involved in the causation of the disease is the relatively high frequency of vaginal infections in such patients. The frequency of cervical carcinoma is reported to be increased in women who have or have had venereal disease (4, 9, 10, 14). In England, Scotland and Wales a covariation has been demonstrated for frequency curves of cervical carcinoma and venereal disease in two cohorts 20 years apart (1). The frequency of cervical carcinoma of the cervix is also reported to be

high in marital clusters (7). The carcinogenic agent common in these studies is not known. Herpes virus (12) might be responsible but so too could be carcinogenic metabolites of bacterial origin.

The present study was designed to get an idea as to how to trap the carcinogenic factor and to elucidate the latency period between infection and development of cervical cancer. We therefore reviewed women who had had gonorrhea 23 or 24 years previously and been repeatedly examined cytologically since then. The occurrence of preinvasive and invasive cervical carcinoma was recorded and compared with the corresponding figures in closely age matched controls.

## PATIENTS AND METHODS

The material consisted of the records of 164 (42.2 per cent) of 389 women with gonorrhea who had been seen in the Department of Gynecology or Department of Venereal Diseases Malmö General Hospital in 1954-55 and who were still living in the area of Malmö which is served by this hospital only. In all of them the diagnosis had been confirmed by culture of material from the uterine cervix, urethra and sometimes from the rectum. From 1956 they had been followed cytologically either by gynecologic practitioners or at the Department of Gynecology at the same hospital. From 1966 they had taken part in mass screening examinations every fourth year. When the vaginal smears showed changes biopsy specimens had been obtained by means of the colposcope. The diagnosis of carcinoma of the cervix was always verified by histologic examination of biopsy specimens or of specimens obtained at conization or hysterectomy.

The controls consisted of 164 closely age matched women who had been examined with cytological smear technique in the same way as the patients with gonorrhea in their history. The frequency of gonorrhea in the control group was calculated to be 0.5-1.0 case according to the statistics for venereal disease in Malmö.

Fisher's exact test was used in the statistical analysis.



Table 1 *Preinvasive and invasive cervical carcinoma in women who had had gonorrhea 24 or 23 years previously (n=164) compared with controls (n=164)*

Stage	Patients	Controls
Ca in situ	29 (17.7)	7 (4.3)
Invasive ca colli uteri	8 (4.9)	1 (0.6)
Total	37 (22.6)	8 (4.9)

Figures within parentheses denote per cent

## RESULTS

Carcinoma *in situ* had developed in 29 (17.7 per cent) of the 164 women who had previously had gonorrhea compared with 7 (4.3 per cent) of the controls ( $p < 0.001$ ). Invasive cervical carcinoma was diagnosed in 8 (4.9 per cent) of the patients against one (0.6 per cent) of the controls ( $p < 0.02$ ). Preinvasive or invasive carcinoma was thus demonstrated in 37 (22.6 per cent) of the patients but in only 8 (4.9 per cent) of the controls (Table 1) which implies a relative risk of 4.6 ( $p < 0.001$ ). All the invasive tumors of the cervix were squamous epithelial lesions. No adenocarcinomas were seen.

The age distribution of the patients at the time of infection with gonorrhea and at the time of diagnosis of carcinoma is given in Table II. The frequency of gonorrhea was highest in the 21–25 age class. The youngest patient was 15 years old at the time of the diagnosis and the oldest 46. The ages of the patients with carcinoma *in situ* ranged from 26 to 60 years (mean 35) at the time of the diagnosis. The corresponding figures for the controls were 29 to 56 (mean 37). Invasive cervical carcinoma was diagnosed in patients between 35 and 60 years (mean 44). The only control who had this advanced stage was 41 years at the time of diagnosis. Of the 8 patients with invasive cervical carcinoma one was in stage 1a, 5 in stage 1b, one in stage 2b and one in stage 4. In the control group the single case of invasive carcinoma was in stage 1b.

Fig. 1 shows the interval between the time of infection with gonorrhea and the diagnosis of carcinoma *in situ* or invasive cervical carcinoma. The mean for carcinoma *in situ* was 11.6 years and for invasive carcinoma 13.5 years. One 51-year-old patient in whom a Papanicolaou smear suggested further investigation was not seen again until she was 60, was then found to have invasive cervical carcinoma in stage 2b.

Fig. 2 shows the intervals between the diagnosis of gonorrhea and of carcinoma *in situ* or invasive carcinoma regardless of age of the patients. The shortest interval for carcinoma *in situ* and for invasive cervical carcinoma was 6 years, the longest 21 and 23 years respectively. The curve shows substantial increase after 11 years. After additional 5 years the frequency of new cases of carcinoma *in situ* decreased while that of invasive carcinoma did not.

Of the remaining 127 women with previous gonorrhea examined but not registered as having cervical neoplasia, there were 5 in whom vaginal smears shown a Papanicolaou score of IV–V and not be persuaded to undergo further investigation. Twenty-seven women had shown changes which only temporary however or which further investigation had shown to be benign. The figures for the control group were 2 and 15 women.

## DISCUSSION

The investigation corroborated the finding in earlier retrospective investigations that cervical cancer is more common among women with vaginal infection in their history. The present study can be considered as a prospective investigation of a well defined clinical material of patients with gonorrheal infection diagnosed at Malmö General Hospital in 1954 and 1955 by culture of material from the cervix, urethra and sometimes also from the rectum. When starting

Table II *Age of patients at diagnosis of gonorrhea and of patients and controls at diagnosis of cervical neoplasia*

Age groups	≤ 15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	Total
Gonorrhea infection	1	45	66	26	8	8	7	3			164
Ca in situ				10 (2)	9 (2)	5 (1)	2 (1)	2		1 (1)	29
Invasive ca colli uteri					2	1	2 (1)	2		1	8

Figures within parentheses denote controls

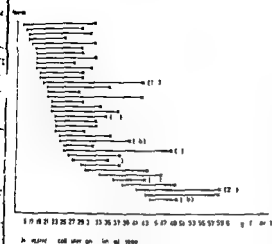


Fig 1 Interval between diagnosis of gonorrhea and carcinoma *in situ* or invasive cervical carcinoma

retrospectively with women in various stages of cervical carcinoma anamnestic data about venereal disease are of doubtful value and are often denied by the patients.

Of the primary material of 389 patients 164 (42.2 per cent) were still alive and residing in the catchment area of the hospital in 1978 when they were reviewed for preinvasive or invasive cervical carcinoma. There is no evidence of any significant difference in general health between the women still living in Malmö and the remainder who had moved from the catchment area and were therefore not included in the material. It is possible, however, that the gynecologic screening of as many as 91.8 per cent of all women up to 69 years of age in Malmö (2) might have resulted in a higher frequency of early diagnosis of cervical carcinoma than elsewhere. This possibility is strengthened by the coincidence of the rise of the curve in Fig. 2 in the 11th year after infection with gonorrhea simultaneous with the inception of the screening project.

The controls consisted of closely age matched women who had participated in the screening in exactly the same way as the women known to have previously had gonorrhea. Since we were unaware whether the 164 controls had previously had gonorrhea or not they may be regarded as a representative sample of the general female population of the town with an anticipated negligible incidence of 0.5–1.0 case in this group.

As long as we do not know the causal factor of carcinoma *in situ* or of invasive cervical carcinoma we

prefer to use the term interval instead of latency period for the time between the diagnosis of gonorrhea and the diagnosis of malignant changes in the portio.

We cannot say anything about the actual causal agent of the malignant process, i.e. whether it could be *Neisseria gonorrhoeae* alone or *N. gonorrhoeae* combined with some other simultaneously acquired infectious agent of bacterial or viral origin (11). Furthermore a carcinogenic factor acquired at the same time as the gonorrheal infection cannot be excluded.

Assuming that the causal agent that started the malignant process was acquired in association with the gonorrhea, the youngest group is the most interesting. In view of their age they could hardly have been exposed to cancerogenic agents for any length of time before they had been infected with *N. gonorrhoeae*. This would mean that in this age class the interval (12.4 years) between the diagnosis of gonorrhea and the detection of carcinoma *in situ* or invasive carcinoma might be a fair estimate of the latency of such lesions.

The arithmetic mean interval between gonorrhea and carcinoma *in situ* was 12.4 years in the 15–20 years group compared with 11 in the remaining patients, i.e. a difference of only 1.4 years. This difference might be explained by genetic liability with increasing age or previous unknown infections. Women who acquired the infection later in life might have been exposed to some causal agent of the malignant process earlier in life. If so, it would explain the

number of cases

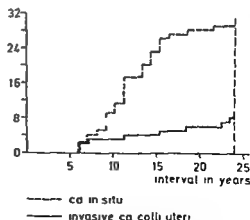


Fig 2 Interval between diagnosis of gonorrhea and carcinoma *in situ* or invasive cervical carcinoma irrespective of age

shortness of the interval between the diagnosis of gonorrhea and of preinvasive or invasive carcinoma of the cervix in such women. Furthermore, one can not exclude the possibility of their having had gonorrhea even on some still earlier occasion.

It was not thought worthwhile to study the effect if any of parity or socio-economic status, as such an investigation would have been very laborious and the results would hardly have warranted any clear-cut conclusion for two reasons: first, substantial changes in socio-economic status during the long period covered by the present study; and second, the increasing frequency of cohabitation. We do not believe that prostitutes were over-represented in our material, but rather that it is an acceptable sample of urban women with gonorrhea in their history.

*Herpes genitalis* and/or other genital infections which may occur alone or combined with gonorrhea may be of importance in the development of malignant lesions. Vaginal smears from a group of 37 women in our hospital who had 1–5 years earlier had virologically verified *herpes genitalis* without coexisting gonorrhea were examined for cervical neoplasia. Cytologically, 35 of the women had a Papanicolaou smear I and 2 a Papanicolaou smear II. In both cases biopsy showed benign atypia (17). This does not mean that herpes is of no noteworthy importance, but the shortness of the interval precludes any definite conclusion as well as any comparison with women who had previously had gonorrhea.

The present results clearly show that the risk of developing malignant lesions in the portio was four times as high in women who had had gonorrhea as in those who had not. This increased risk is sufficient to warrant particular attention at the gynecologic examination of such women.

The similarity in age distribution regarding the occurrence of cervical neoplasia between patients and controls suggests that gonorrhea is associated with a carcinogenic agent, but less likely with a promoter agent.

In search for the carcinogenic agent of carcinoma of the uterine cervix, one might expect a better chance of finding this agent at the time of infection than when a carcinoma of the uterine cervix has already reached a preinvasive or invasive stage.

#### ACKNOWLEDGEMENTS

We are grateful to Bo Gullberg, Institute of Statistics, University of Lund, for valuable help with the statistical analyses.

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Submitted for publication January 28 1980

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## SEROUS PAPILLARY NEOPLASMS ARISING IN PARAMESONEPHRIC PAROVARIAN CYSTS

A report of eight cases

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**Abstract:** Eight cases of papillary serous cystadenoma arising in paramesonephric parovarian cysts are presented. Some of these benign lesions were diagnosed preoperatively as such and were only detected pathologically. In only one case was the tumor-bearing cyst symptomatic owing to its large size. The etiology, pathogenesis and evolution of this lesion are briefly discussed.

Parovarian cysts of paramesonephric origin are very common but rarely give rise to clinical complications. One of the rarest complications of these cysts is the secondary development of an intracystic papillary tumor, benign or malignant (8). These tumors are so rare that, with one exception (4), they have been documented as individual case reports (5-7). So far only one carcinoma arising within a paramesonephric cyst of the parovarium is on record (1), while there is one report of a carcinoma originating from parovarian mesonephric rests (9).

Secondary neoplasia developing within paramesonephric parovarian cysts has not been investigated systematically. This report documents a systematic study of all parovarian cysts removed surgically or submitted along with excised adnexae. Over a three-year period eight papillary tumors arising in paramesonephric parovarian cysts were seen.

### MATERIAL AND METHODS

The study material, obtained from women ranging in age from early twenties to late seventies, was heavily biased in favor of young women aged twenty to forty. It consisted of 194 pairs of fimbriated distal tubal segments removed for sterilization and of 308 uteri bearing one or two attached tubes. Eleven parovarian cysts were submitted separately.

All distal tubal segments removed for tubal sterilization and all tubes along with excised uteri were carefully scrutinized for visible paratubal (parovarian) cysts. Their location

(8) was identified as fimbrial (corresponding to the hydatid cyst of Morgagni), infratubal or inferior (lying within the concavity of the distal ampulla), supratubal or superior (lying subserosally on the rounded superior aspect of the tube). Their number, size, shape, color, consistency were noted. They were then opened and their inner walls were carefully inspected for opaque areas or foci of visible intracystic papillary ingrowths. The cysts were abundantly sampled for histology and all areas of thickening or intracystic papillary growth were processed in toto. The parovarian cysts separately submitted were studied in the same way and appropriate samples taken. The paraffin-embedded sections were stained with hematoxylin and eosin and the special stains used on selected cases included the Masson Trichrome for the differentiation of smooth muscle and fibrous tissue, the PAS/PAS-Diastase combination for glycogen and the PAS-Alcian Blue stain for the detection of neutral and acid mucopolysaccharides and the delineation of basement membrane.

### RESULTS

Parovarian cysts were identified in nearly 92 per cent of the cases studied and the infratubal variety was by far the commonest, with the fimbrial cyst being a poor second. These round or ovoid cysts, ranging in size from 1 mm to 12 cm, were lined by a smooth thin translucent wall traversed by many stretched blood vessels. In eight cases (Table I) the typically smooth inner lining was variably coated by single or multiple closely packed clusters of firm, greyish white verrucous ingrowths (Fig. 1). These clusters, ranging in size from 2 mm to 3.7 cm and in height from 1 mm to 1.8 cm, were easily detected on the inner cyst lining and, when large enough, could also be palpated on the outside as focal thickenings of the wall.

Histologically, the diagnosis of papillary serous cystadenoma (Figs. 2 and 3) was based on the presence of closely packed, predominantly fibrous or edematous papillary ingrowths lined by low cuboidal tubal epithelium continuous with a similar epithelium

Table 1

Patient	Age (years)	Clinical data	Relevant pathological findings
1	33	Elective tubal sterilization by distal salpingectomy	Fimbria and ampulla of right tube arched over infratubal intraligamentous cyst measuring 6 × 4 × 5 cms. Tense cyst filled with clear fluid lined by 1–2 mm thick translucent wall with few clustered tiny shallow endophytic growths <i>Histology</i> Papillary serous cystadenoma arising in paramesonephric cyst
2	29	Postpartum tubal sterilization by distal salpingectomy	Two cysts in mesosalpinx. Larger infratubal para-ampullary cyst measuring 6 mm across filled with thick jelly like pale yellow fluid lined by 1 mm thick translucent wall with small foci of opacity but no definite papillae <i>Histology</i> Microfocal papillary serous cystadenoma in paramesonephric cyst
3	28	Cesarean section for cervical dystocia Tubal sterilization by distal salpingectomy	Two intraligamentous infratubal para-ampullary cysts. Larger cyst measuring 15 mm across filled with clear fluid lined by smooth 1 mm thick translucent wall with tiny clusters of grape like sessile papillary ingrowths <i>Histology</i> Papillary serous cystadenoma in paramesonephric cyst
4	33	Elective tubal sterilization by distal salpingectomy	a) Thick walled opaque infratubal intraligamentous cyst measuring 15 × 12 × 6 mm and lying inferior to ampulla of right tube lined by smooth wall with many clustered finely granular verrucous ingrowths <i>Histology</i> Papillary serous cystadenoma in paramesonephric cyst b) On left side similar opaque cyst measuring 3 cms in diameter and showing extensive fine granularity of inner wall. <i>Histology</i> Papillary serous cystadenoma in paramesonephric cyst
5	41	Elective tubal sterilization by distal salpingectomy. Pelvic floor repair for prolapse	Left fimbrial cyst measuring 5 mm across filled with clear fluid lined by 1 mm thick translucent wall with few finely granular ingrowths <i>Histology</i> Papillary serous cystadenoma in paramesonephric cyst
6	4	Ruptured left ectopic pregnancy left salpingectomy	Infratubal parovarian cyst 1 cm across and containing inspissated jelly like yellowish green material with few barely visible asgrowths <i>Histology</i> Early papillary serous cystadenoma in paramesonephric cyst
7	41	Elective tubal sterilization removal of distal right tube with attached cyst excision of segment of left tube (Pomeroy technique)	Infratubal parovarian cyst measuring 3.5 cms and containing clear slightly viscous fluid. Single cluster of firm greyish papillary ingrowths <i>Histology</i> Papillary serous cystadenoma in paramesonephric cyst
8	31	Lower abdominal and pelvic pain for four weeks Clinical diagnosis ovarian cyst Operative findings large right parovarian cyst impacted in pelvis enlarged edematous cystic right ovary separate from cyst left ovarian cyst	a) Right parovarian cyst measuring 12 cms across and weighing 500 grams closely applied to stretched tube. Cyst made up of two widely communicating locules filled with clear fluid lined by smooth translucent wall with two small foci of fine verrucous endophytic growths <i>Histology</i> Papillary serous cystadenoma in paramesonephric cyst b) Right ovary enlarged with marked edema and follicular cysts c) Left ovarian cyst intracystic papillary serous cystadenoma

covering the flat surface of the cyst wall. The serous nature of the cyst was established by its histologic and tinctorial similarities to tubal epithelium i.e. the presence of scanty glycogen and variable amounts of adluminal neutral and acid mucopolysaccharides. The cyst was identified as paramesonephric and parovarian by its epithelium which was tubal histologically and tinctorially (Fig. 4) by the lack of smooth muscle and ovarian tissue in its fibrous wall and by the absence of thin shallow intracystic tubal fronds typically seen in accessory tubes or ostia.

Case 8 was exceptional in being clinically symptomatic by virtue of its size. Pathologically the cyst differed from the others only by its unusually large size (diameter = 12 cm and weight = 500 g).

## DISCUSSION

These cases fulfil the criteria of papillary cyst adenoma arising within paramesonephric parovarian cysts and closely resemble the previously reported cases (5, 7, 8). These tumors though similar to their more common ovarian counterparts are fairly characteristic in showing only patchy involvement of the inner cyst wall (usually less than 20 per cent of its surface area) and a tendency to close packing of swollen bulbous papillae lined by low cuboidal tubal epithelium and subtended by edematous or fibrous hypovascular cores.

Three possible sites of origin for this lesion deserve consideration. The most likely site of origin is clearly the pre-existing parovarian cyst which in develop-

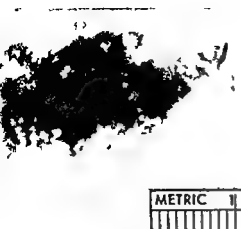


Fig 1 Gross photograph of firm velvety closely packed verrucous growths typical of papillary serous cystadenoma in parovarian cyst. Note translucent wall with stretched blood vessels



Fig 3 Closely packed bulky edematous and fibrous papillae lined by low cuboidal epithelium (H & E  $\times 80$ )

of tubal origin and is frequently seen at all ages (8). This suggestion is supported by the strict localization of these intracystic tumors near the tubal ostium and the developmental paramesonephric cysts are present and also by the identity of these tumor bearing ovaries and their non neoplastic counterparts. The second site an endosalpingeal cyst (2) arising within the accessory or supernumerary ovary is not supported by the consistent absence of ovarian tissue in the cyst wall. A third possibility (5-7) i.e. origin from an endosalpingeal focus in the parovarium we consider unlikely since the strict confinement of

these tumors to the para-ampullary and para-ovarian tissues does not correspond to the widespread distribution of endosalpingiosis from the tubal ostium to the cul-de-sac (6).

Our systematic study which only uncovered eight cases of this tumor in a series exceeding 2,200 cases confirms the rarity of this secondary complication of these parovarian cysts and does not justify their routine en passant removal during pelvic surgery. However, since there is a tendency for these lesions to occur in larger cysts and since their full neoplastic potential is still unknown, it might be wise to remove



Fig 2 Parovarian cyst with focal papillary cystadenoma (short arrow) and fibrous wall. Note adjoining tubal fimbria (long arrow) (Masson Trichrome  $\times 52$ )



Fig 4 Flat non neoplastic lining of parovarian cyst showing features of tubal epithelium i.e. occasional clear ciliated cells (arrow) and abundant secretory cells with adluminal deposits of mixed neutral and acid mucopolysaccharides (PAS-Alcian Blue  $\times 890$ )

the large asymptomatic ones especially as they can unpredictably expand and cause clinical problems.

The etiology and pathogenesis of secondary neoplasia arising in parovarian cysts remain unknown and careful review of the clinical data of our small series of patients has yielded no meaningful information. Bilaterality concurrence with a similar intra-ovarian cystadenoma and a predilection for occurrence in the most common infratubal intraligamentous cyst have been noted but the series is too small for significant deductions. The predominantly small size of these secondary tumor foci within these parovarian cysts, their inactive looking low cuboidal epithelium and the fibrous or fibrocalcific regression of their bulky cores suggest that these tumors tend towards spontaneous regression rather than progression to malignancy which appears to be exceptional (1). It is noteworthy that the eight documented cases of ovarian cancer exhibiting spontaneous regression have been papillary serous cystadenocarcinomas (3).

These tumor bearing cysts are clinically indistinguishable from their non-neoplastic counterparts and when symptomatic the signs and symptoms are due entirely to cyst size. The intracystic tumor cannot therefore be diagnosed preoperatively but at operation its presence can be suspected if the typically thin translucent cyst wall shows focal or patchy opacity and thickening. It is hoped that this report will stimulate interest in this lesion and allow the collection of enough data for meaningful analysis.

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*Submitted for publication December 29 1978*

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## PROBABLY VIRUS-INDUCED EPITHELIAL LESIONS IN PREINVASIVE CERVICAL CANCER

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**Abstract** There is an urgent need to approach the evaluation and classification of cervical intraepithelial lesions and their cytopathological correlations as specifically as possible. Preinvasive cancer (differentiated and undifferentiated carcinoma in situ) of the uterine cervix the most advanced stages of cervical intraepithelial neoplasia should be clearly defined and described and the diagnostic and therapeutic management including the follow up of the patients outlined in detail.

The present results based on a material of 524 cone biopsies clearly indicate the existence of two different types of probably virus-induced epithelial lesions in the two histopathologic groups of preinvasive cancer of uterine cervix.

Cervical neoplasia probably develops as a result of a combination of initiating events and promoting agents. Virus infections sexually transmitted from male to female and inducing epithelial lesions might be such an initiating event and even a promoting agent.

Rawls *et al* (12) discuss the role of virus infection in the etiology of cervical neoplasia and stress that a virus might act as a carcinogen or co-carcinogen or that a virus infection may follow a neoplastic transformation which indicates a greater affinity of cancerous tissue for the virus. Meisels *et al* (6) found a surprisingly high occurrence of condylomatous lesions a venereally transmitted viral infection which at times had been interpreted as dysplasia of the uterine cervix. In view of the fact that condylomata acuminata have been proved to undergo malignant transformation and that they behave epidemiologically in a way similar to carcinoma of the cervix the authors speculate that these viral infections might represent a precursor of cervical neoplasia. They state that condylomatous viral lesions are generally present in a younger group than dysplasia and carcinoma in situ (CIS). Both condylomata and cervical neoplasia are directly related to sexual promiscuity and an early onset of sexual activity. It is conceivable that the im-

mature cervix when still covered by a fragile single layer of epithelial cells could be more vulnerable than the mature cervix.

Naib *et al* (8, 9, 10) discuss the relation of cyto- and histopathology of genital herpes virus infection to cervical neoplasia. The authors analysed 673 cytologically detected cases of herpes genitalis with regard to the association of histologically confirmed cervical neoplasia. Among these 673 cases there were 69 women with dysplasia, 25 with CIS and 11 with invasive cervical cancer, a total of 105 cases (15.6 per cent) of neoplastic and malignant epithelial changes in the uterine cervix. The authors reported data from serological studies which indicate that the herpetic infections were not newly acquired but instead represented recurrences of latent herpes. The initial event probably having occurred months or even years prior to the detection of the cervical neoplasia. The age prevalence of genital herpes was found to be 5 years earlier in the study group than that of cervical dysplasia and 10 to 15 years earlier than that of CIS.

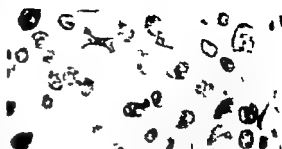
Tobin *et al* (15) described an animal model that demonstrates the development of varying degrees of basal cell hyperplasia, metaplasia and dysplasia following herpes virus infection. Although the study spanned a period equivalent of 60 human years there was no evidence of anaplasia. The authors discuss the role of herpes virus as an initiator in the process which leads to cervical carcinoma.

As important as studies on the relation of genital virus infection to cervical neoplasia is the evaluation of the occurrence of histopathologically confirmed probably virus induced epithelial lesions close to or within foci exhibiting dysplasia or CIS (14) and their early detection by means of cervico-vaginal smears. According to Naib *et al* (10) hypertrophy of the cytoplasm and nucleus of infected cells is usually observed in viral infections. The cells increase in size and show nuclear and cytoplasmic edema as indicated by the frequent appearance of perinuclear halo.





Fig 1 and 2 A probably virus induced epithelial (Type II) lesion is located between normally differentiated squamous epithelium (to the right) and neoplastic epithelium (to the left). The lesion shows hypertrophy of the cytoplasm and



nucleus with perinuclear halo and in many cells the nuclear chromatin is displaced to the periphery ( $\times 175$  and  $\times 640$  respectively)

These are a result of the shrinkage of the enlarged nucleus and cytoplasm due to dehydration of the cell during fixation and staining. The nucleoli also enlarge and become vacuolated. The nuclear chromatin is displaced to the periphery and results in central nuclear clearing. Multinucleation is also seen. Granules appear in the centre of the nucleus and form an intranuclear inclusion surrounded by a more or less prominent halo. Cells infected by virus exfoliate easily and usually appear abundant in cervical smears when they show degenerative changes in the cytoplasm and nuclei with vacuolization, ballooning and bizarre shapes.



Fig 3 and 4 An area of probably virus induced epithelial (Type II) lesion is located within preinvasive cancer contrasting with the neoplastic squamous epithelium of small and dark undifferentiated cells. Note hypertrophy of the

## MATERIAL AND METHODS

In the present study 524 cervical cone biopsies, 471 with preinvasive cancer (differentiated and undifferentiated CIS) and 103 with dysplasia in women 16–39 years of age were evaluated histologically as well as cytologically in order to investigate the occurrence of probably virus induced epithelial lesions.

The cones were fixed in formalin, dehydrated in paraffin. For investigative purposes 10 to 15 serial sections were embedded in paraffin blocks, sectioned and stained by the hematoxylin-eosin and van Gieson techniques.

The cytological smear, 3 to 7 specimens from each case investigated, were collected from the cervix and vagina, fixed and stained according to the original Papanicolaou technique (11). The histological as well as the cytological material was handled by the staff of the University Hospital, Uppsala, during 1970–79.



cytoplasm and nucleus edema and perinuclear halos. The nuclei are dense and the nuclear chromatin displaced to the periphery ( $\times 175$  and  $\times 640$  respectively)

## RESULTS

is differentiated preinvasive cancer the normal granified structure of squamous epithelium is no longer recognizable. The basal cell layers consist of undifferentiated neoplastic cells with small and dark uniform nuclei and indistinct cell borders. The middle and especially the upper cell layers show larger neoplastic cells and the degree of differentiation may vary from incipient to moderate while the cell borders are distinct. In all cell layers there is considerable variation in the size and shape of the cells and the nucleo-cytoplasmic ratio is significantly increased as to within malignant limits. Mitoses and nuclear pleomorphism are usually found throughout the lesion.

In undifferentiated CIS dark undifferentiated cells with indistinct cell borders are found throughout the lesion. The nuclei are crowded and vary in size and shape. Areas with bizarre nuclear enlargement create a highly malignant pattern of the lesion and typical atypical mitoses are often seen in the superficial layers.

The cellular pattern of differentiated CIS shows incipient or distinct differentiation. The nucleo-cytoplasmic ratio is greatly increased. There is considerable variation in the nuclear size and shape and hyperchromatic and irregular nuclei are coarse nuclear or filamentous in their chromatin pattern. Areas of differentiated neoplastic cells are frequent and some of the squamous cells show only incipient differentiation with no differentiation of the basal cell layers.

The cells of undifferentiated CIS are small and dark with scanty cytoplasm and a greatly increased nucleo-cytoplasmic ratio. The cytoplasmic borders are indistinct. The nuclei are prominent and show pleomorphism, anisonucleosis and hyperchromatism with coarse clumping of the chromatin. The nuclear borders are irregular and the nucleoli vary in number, size and shape.

In dysplasia the basal cell layers consist of small dark undifferentiated neoplastic cells similar to those of undifferentiated or differentiated CIS. The cells of the lower middle layers consist of larger cells showing incipient differentiation and dark nuclei and the upper middle cell layers exhibit fairly well differentiated squamous cells which are larger and fewer towards the surface. The nucleo-cytoplasmic ratio is significantly lower in the middle cell layers than in the basal cells and pleomorphism in nuclei as well as mitoses are significantly less frequent. The superficial

cells are well differentiated normal or only slightly atypical flattened squamous cells.

In the present material 421 cone biopsies were evaluated with respect to the occurrence of histologically confirmed probably virus induced epithelial lesions close to but unconnected with preinvasive cancer or within preinvasive cancer. Two basic patterns were found.

Areas of probably virus induced epithelial lesions were found to be located close to but without any connection with squamous epithelium exhibiting preinvasive cancer (Type I). These well demarcated areas showed hypertrophy of the cytoplasm and nucleus and larger cells with nuclear and cytoplasmic edema. Because of the dehydration of the cell during fixation and staining a perinuclear halo is observed and the nuclear chromatin is displaced to the periphery. Multinucleation and intranuclear inclusions are also seen in these epithelial areas. As a rule there is a sharp border between the deep denser cell layers and the superficial layers consisting of clear cells with degenerating nuclei surrounded by a clear halo that may occupy most of the cell (Fig 1-2).

Areas of probably virus induced epithelial lesions located within preinvasive cancer as sharply demarcated light areas of squamous epithelium are considered to belong to a special group of these changes (Type II). These sharply demarcated foci contrast with the neoplastic squamous epithelium which consists of small and dark undifferentiated cells or cells exhibiting incipient or moderate squamous differentiation (Fig 3-4).

Table I shows that in 51 out of 421 investigated cases (12.1 per cent) a probably virus induced and morphologically clearly recognizable and definable lesion was found in 26 (6.2 per cent) without any connection to preinvasive neoplasia. In 25 cases (5.9 per cent) probably virus induced lesions were within areas of preinvasive carcinoma. Thus the total number of probably virus induced lesions within preinvasive carcinoma seems to be about the same as in those lesions not connected with CIS.

The cytopathologic pattern of preinvasive cancer was investigated in the present study by evaluating the occurrence of benign cells (normally differentiated squamous cells, metaplasia, inflammatory changes), dysplastic (dyskaryotic) squamous cells and malignant cells exhibiting squamous differentiation of high, moderate or low degree. In all a series of cervico-vaginal smears from 128 women with preinvasive cancer was investigated and correlated with

Table I Incidence of probably virus induced lesions in 421 cones with preinvasive cervical cancer

	CIS Number of cones		Total
	Differentiated	Undifferentiated	
Viral lesion Type I	10 ( 8.7)	16 ( 5.2)	26 ( 6.2)
Viral lesion Type II	17 (14.8)	8 ( 2.6)	25 ( 5.9)
CIS only	88 (76.5)	282 (92.2)	370 (87.9)
Total	115	306	421

the figures within parentheses denote number in per cent

series of dysplasia in 103 women in the same groups. The results are shown in Table II.

Table II shows that the screening results in cases of preinvasive cancer were good: there were only 3 cases of false negative results (2 per cent). The results in cases of dysplasia were significantly different: with 8 per cent (9 cases) false negative and 49 cases (48±5 per cent) classified as CIS. A significant number (see Discussion) of these inaccurate results was induced by a false interpretation of probably virus induced lesions.

## DISCUSSION

In the early detection of cervical neoplasia there is a tendency to classify an increasing number of cases exhibiting more or less total replacement of squamous epithelium by atypical but not necessarily neoplastic or malignant cell layers as preinvasive cancer.

It is important to recognize the typical and different morphological pattern of probably virus induced lesions in squamous epithelium described in detail by several authors (6, 7, 8, 9, 10, 15) in histologic sections as well as in cervico vaginal smears.

The probably virus induced epithelial lesions are located in the cervical canal either close to but uncon-

nected with areas of preinvasive cancer or within the cervical neoplasia and are seen as well demarcated pale areas exhibiting a typical morphologic pattern.

Reviewing smears previously diagnosed as representing patterns of mild dysplasia, Meisels *et al.* (7) found that 70 per cent of the cases were consistent with condylomatous changes induced by the venereally transmitted virus of condyloma acuminatum. The cytologic patterns of these lesions were described in detail by Meisels and Fortin (6). Dyskeratosis condensed and hyperchromatic nuclei, perinuclear clearing, multinucleation and inclusion bodies are cytologic changes that might be interpreted as corresponding to cervical neoplasia.

The progression of premalignant cervical lesions into CIS and invasive cancer is of great importance especially in the younger age groups. On the basis of the extensive studies of Barron and Richart (1) and Fidler *et al.* (4) it can be concluded that 50 per cent of patients with various forms of untreated dysplasia progress to CIS while 28 per cent remain in the stage in which they were detected, 16 per cent progress to a higher stage of dysplasia and only 6 per cent show spontaneous regression. Finally, an unknown number of cervical premalignant neoplastic lesions probably progress directly to invasive cancer.

The pattern of progression and regression of probably virus induced epithelial lesions in the uterine cervix is unknown and should carefully be studied cytologically and histologically in large groups of women. The second important question concerning probably virus induced cervical lesions is the therapeutic management of these cases. The basic difficulty in answering this question is that these lesions are often found together with classical dysplasia or CIS.

In the present study cases with dysplasia could not be included due to the fact that these women as a rule are not investigated and treated by conization following initial cervical biopsies. The 103 cases of dysplasia investigated were selected according to age

Table II Cytopathology of preinvasive cancer (128 cases) and dysplasia (103 cases)

Cytology	Histology	
	CIS	Dysplasia
Normal differentiation		
metaplasia inflammation	3 ( 2)	9 ( 8)
Dysplasia	24 (19)	45 (44)
Preinvasive cancer	101 (79)	49 (48)
Invasive cancer	—	—
Total	128	103

the figures within parentheses denote number in per cent

and parity (higher than the average woman) and therefore the true frequency of probably virus induced cervical lesion is not known. However the cytologic pattern can be evaluated and correlated with preinvasive cancer. A significant number of the 49 cases (48±5 per cent) cytologically classified as preinvasive cancer was overdiagnosed due to the occurrence of cells or groups of cells in the cervico-vaginal smears exhibiting a typical pattern of viral infection.

In 51 out of 421 morphologically investigated cases (12.1 per cent) a probably virus induced epithelial lesion was found. The number of lesions within preinvasive carcinoma seems to be about the same as those lesions not connected to CIS.

Czernobilsky *et al* (2) discuss the prevalence of inflammatory and proliferative cervical lesions in Jewish and non Jewish women. The authors found that in spite of the low incidence of cervical cancer in Jewish patients the prevalence of cervical inflammatory and non malignant proliferative epithelial changes corresponded to the number found in non Jewish populations. According to the authors there seems to be two possible explanations for their findings: first the histologic spectrum leading to CIS stops at the dysplastic stage or secondly dysplasia may in fact represent a non specific reaction which is not necessarily a stage in the process leading to CIS or invasive cancer of the uterine cervix.

Meisels *et al* (5) discuss the role of age at first coitus and the use of oral contraceptives as epidemiological aspects in the development of dysplasia of the uterine cervix. The authors conclude that in homogeneous populations highly significant correlations were found between the early onset of sexual activity and the occurrence of dysplasia and oral contraceptive use and the occurrence of dysplasia. The authors stress that dysplasia behaves epidemiologically essentially as a venereal disease like CIS and invasive cancer and that it remains to be seen whether all dysplasias form one continuum or several different forms exhibiting different rates of progression.

Discussing the surprisingly high rate of condylomatous lesions in young women Meisels *et al* (7) speculate that these venentially transmitted viral infections might represent a precursor of cervical neoplasia. The possible role of herpes virus as an initiator in the process leading to cervical carcinoma is discussed by several authors (8, 9, 10, 12, 15).

One approach is to consider cervical intraepithelial neoplasia a spectrum of one and the same disease be-

ginning from mild dysplasia and ending with undifferentiated CIS. According to Richart (13) and Ferenczy (3) there is a logarithmic increase in the number of undifferentiated basal cells with increasing severity of this disease.

There is an urgent need to approach the evaluation and classification of cervical intraepithelial lesions and their cytopathologic correlations as specifically as possible. Differentiated and undifferentiated preinvasive cancer of the uterine cervix, the two most advanced stages of cervical intraepithelial neoplasia should be clearly defined and described and the diagnostic and therapeutic management including the follow up of the patients outlined in detail.

The present results based on series of 524 cone biopsies clearly indicate the existence of two different types of probably virus induced epithelial lesions in the two histopathological groups of preinvasive cancer of the uterine cervix.

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Czernobilsky *et al* (2) discuss the prevalence of inflammatory and proliferative cervical lesions in Jewish and non Jewish women. The authors found that in spite of the low incidence of cervical cancer in Jewish patients the prevalence of cervical inflammatory and non malignant proliferative epithelial changes corresponded to the number found in non Jewish populations. According to the authors there seems to be two possible explanations for their findings: first the histologic spectrum leading to CIS stops at the dysplastic stage or secondly dysplasia may in fact represent a non specific reaction which is not necessarily a stage in the process leading to CIS or invasive cancer of the uterine cervix.

Meisels *et al* (5) discuss the role of age at first coitus and the use of oral contraceptives as epidemiological aspects in the development of dysplasia of the uterine cervix. The authors conclude that in homogeneous populations highly significant correlations were found between the early onset of sexual activity and the occurrence of dysplasia and oral contraceptive use and the occurrence of dysplasia. The authors stress that dysplasia behaves epidemiologically essentially as a venereal disease like CIS and invasive cancer and that it remains to be seen whether all dysplasias form one continuum or several different forms exhibiting different rates of progression.

Discussing the surprisingly high rate of condylomatous lesions in young women Meisels *et al* (7) speculate that these venereally transmitted viral infections might represent a precursor of cervical neoplasia. The possible role of herpes virus as an initiator in the process leading to cervical carcinoma is discussed by several authors (8, 9, 10, 12, 15).

One approach is to consider cervical intraepithelial neoplasia a spectrum of one and the same disease be-

ginning from mild dysplasia and ending with undifferentiated CIS. According to Richart (13) and Ferenczy (3) there is a logarithmic increase in the number of undifferentiated basal cells with increasing severity of this disease.

There is an urgent need to approach the evaluation and classification of cervical intraepithelial lesions and their cytopathologic correlations as specifically as possible. Differentiated and undifferentiated preinvasive cancer of the uterine cervix, the two most advanced stages of cervical intraepithelial neoplasia should be clearly defined and described and the diagnostic and therapeutic management including the follow up of the patients outlined in detail.

The present results based on series of 524 cone biopsies clearly indicate the existence of two different types of probably virus induced epithelial lesions in the two histopathological groups of preinvasive cancer of the uterine cervix.

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Fig 1 Normal colpo-cysto-urethrography a at rest b during cough c voiding d post micturition

One of the groups bladder base insufficiency (b b i) has previously been described by Olesen (9) and in further detail by Olesen & Walter (12). The characteristic morphology is shown in Fig 2. The urethro vesical junction is situated lower and more anterior than normally. The resting bladder base thus becomes funnel shaped with absence of the normal flat horizontal area in front of the internal urethral orifice. The shape and position of the vagina is normal. Clinically this group carries a high incidence of stress incontinence 83.6 per cent. Micturition pressures are slightly lower than normal. The flows vary

from subnormal to high even in the same patient. This variability is characteristic while average flow values are normal.

Another group presents with a normal resting bladder but during cough the bladder base assumes the same characteristics as described above for b b i (Fig 3). Stress incontinence is a complaint in 82.3 per cent of these cases. Forty four per cent showed the same micturition pattern as in b b i during the urodynamic examination while 56 per cent showed either normal or straining micturition.

The third group shows more extensive morpholog

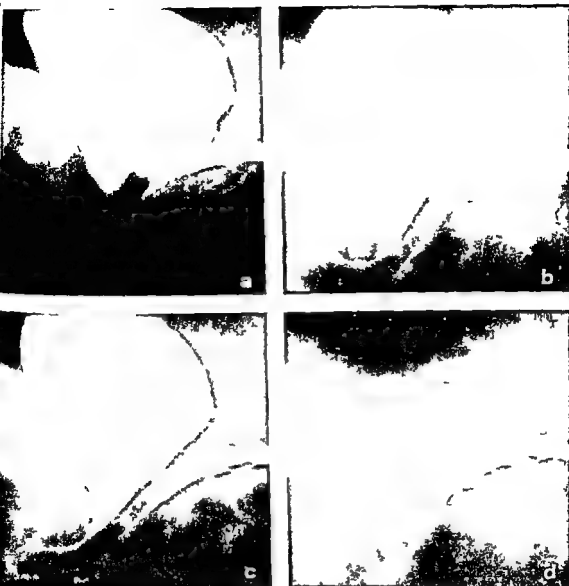


Fig 2 Bladder base insufficiency (b b 1) a at rest b during cough c voiding d post micturition

cal pathology the resting bladder is normal. During cough, however, the entire bladder base slides down in front of the vagina. The axis and position of the vagina is normal, but the anterior vaginal wall slides down in an anterior direction together with the bladder base presenting an eversion of the anterior vaginal wall in the introitus of the vagina (Fig 4). This we have named a prevaginal or anterior bladder descent (a d). Stress incontinence was present in 80.8 per cent of these patients. The urodynamic pattern was different from the preceding groups since 57 per cent used straining resulting in undulating pressure—and

flow curves. Forty three per cent presented either normal or b b 1 patterns during micturition.

These three groups comprised 198 patients and constituted 47.1 per cent of the total number of investigated patients. (The remaining 222 patients presented morphological pictures ranging from normal to severe displacements of bladder and vagina in a postero-inferior direction: trigonocoele and posterior bladder descent, and also included the neurogenic dysfunctions. Among these patients 36.5 per cent complained of stress incontinence. The urodynamic pattern varied according to the various morpholog-



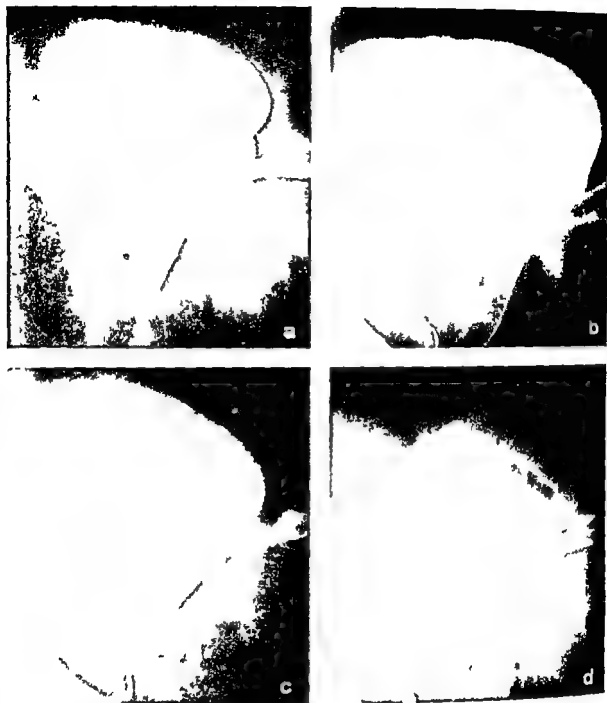


Fig 3 Bladder base insufficiency visible during cough only a at rest b during cough c voiding d post micturition

ical and functional disorders. They will be dealt with in a separate paper.)

In Table I the patients are divided into two groups depending on whether the resting bladder was normal or presented b b 1. Each of these groups is subdivided according to morphology during cough. These four subgroups are further divided according to micturi-

tion morphology. Three micturition patterns (i.e. normal b b 1 and a d) are represented in each subgroup. Four patients could only be classified according to the resting picture and the micturition picture because of unsatisfactory cough radiographs. Eighteen patients were not classified because of complicating posterior bladder descent during micturi-



Fig 4 Two cases of anterior bladder descent a b and c patient displaying b b<sub>1</sub> at rest and anterior descent during cough and micturition a<sub>1</sub> b<sub>1</sub> and c<sub>1</sub> patient with normal

tion. Three patients did not micturate during the examination and one had a urethral stricture.

Twenty-two patients presented the urodynamic pattern of decreased urethral resistance as described by Palm (13). Eight of these showed normal voiding morphology, 7 presented b b<sub>1</sub> and 7 a d morphology during micturition.

## DISCUSSION

The percentage of women with stress incontinence is very uniform in the various subgroups. The morphological differences, however, are important. If the resting bladder is normal and the b b<sub>1</sub> configuration is provoked only by coughing, the patient exhibits the lowest degree of pathology and is labelled grade I. If the resting picture shows b b<sub>1</sub>, the case is marked grade II. All cases that show an a d pattern during

resting bladder displaying anterior descent during cough and micturition.

Table I 198 patients with anterior bladder suspension defects grouped according to morphology of resting cough and micturition pictures

ST NO	NOR										NO M L
COUG	b.b.					a.d.					7
M CTU / TIO	NORMAL					NO MA					a.d.
NO OF T TYS	38	9				75	9	80	100		
ST ESS INC %	84	73	89			75	9	80	100		
SEAS COMP CATE RI POSTE ION DESC NOUS											
2 OF NO M CTU											
ESTING	A										
COUG	b.b.					a.d.					
M CTU / TIO	NORMAL					NORMAL					a.
NO OF T TYS	22	9				3					
ST ESS INC %	77		80			00	00	00			
CASES COMPLICATE WITH POSTE ION DESC NOUS											
1 HAD A URETHRAL S ACTUP DID NOT MICTURATE											

## A. TE OR BLADDER SUSPENSION DEFECTS

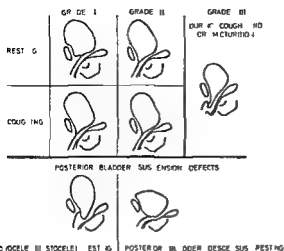


Fig 5 See text for further explanation

cough and/or micturition must have more extensive pathology as the anterior vaginal wall is involved in the displacement. The vaginal attachment to the endopelvic fascia must be insufficient. It is therefore reasonable to group these cases as showing the severest degree of pathology grade 3 of the anterior bladder suspension defects. Fig 5 shows the anterior suspension defects in the three grades as opposed to the posterior bladder suspension defects.

This grouping in various grades allows a precise description of underlying pathology and the degree of involvement of the supporting tissues. Thus it may be used as an aid in the choice of corrective surgical procedure.

Description of the low positioned funnel shaped bladder base is widespread in the literature on urinary stress incontinence (1, 5, 6). Green (4) in a paper on stress incontinence described a type I which corresponds to b b 1 and a type II which includes a d but also includes all other cases of bladder descent. Colpo cysto urethrography by visualization of the vagina enables us to distinguish a d from posterior descent in which the posterior support i.e. the levator muscles are insufficient and the vagina therefore shows a curved posterior displacement. Bethoux *et al* (2) also make this distinction by means of colpo cystograms. They find that stress incontinence is typically linked with a normal position of the vagina. From our study it cannot be said that stress incontinence is specific to this group. Stress incontinence is found in all groups as a fairly common symptom but it is far more typical in anterior bladder suspension defects than in the remaining groups.

## UNDERLYING PATHOLOGY

The bladder displacement is in anterior and inferior direction. Olesen & Grau (10) suggested that the structures which prevent this displacement of the bladder base must be the ligaments in the pelvic fascia i.e. the arcus tendineus fasciae pelvis which pass in a dorsal direction from the bladder neck to which they are firmly connected.

On the basis of these anatomical studies seven patients complaining of stress incontinence and presenting grade 1 and transitional forms between grade 1 and grade 2 have been operated on using a procedure aimed at tightening the arcus tendineus via a supra pubic approach. The bladder neck and the fibroelastic nodes on its lateral aspects described by Zacharin (15) were dissected. From here the arcus tendineus fasciae pelvis was followed posteriorly for about 5 cm. Fig 6 shows the anatomy and the principle of the operation. The structures were clearly definable in all instances. By tightening the arcus tendineus on each side and approximating the two fibroelastic nodes in front of the bladder neck the normal shoulders of the bladder base were created. These shoulders correspond to the flat part of the base in

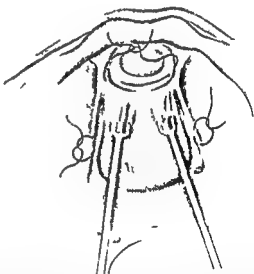


Fig 6 Drawing of operative method. Symphysis pubis upwards. Bladder retracted displaying in front the bladder neck and the fibroelastic nodes connected to the symphysis pubis by the pubo-vesical ligaments. From the fibroelastic nodes the arcus tendineus fasciae pelvis passes in a postero-lateral direction. Tightening sutures are placed in each arcus tendineus and one between the two nodes in front of the bladder neck thus tightening the support around the anterior part of the bladder neck.

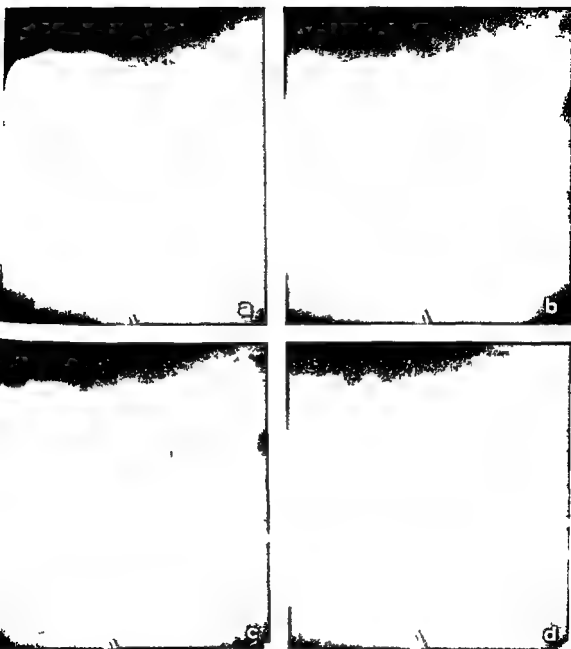


Fig 7 Same patient as Fig 2 after operative tightening of the anterior support of the bladder neck - now normalized a at rest b during cough c voiding d post micturition

front of the internal orifice seen on the radiographs. The dissection was troublesome and gave considerable bleeding so the operation is not suitable for routine use.

Two patients did not agree to a follow up examination. In the remaining five patients stress incontinence ceased. In one patient symptoms suddenly re-

appeared after 11 months and follow up examination revealed that the preoperative conditions had recurred. Four patients suffered from urgency in varying degrees for about a year after the operation but are now at 18 months nearly symptom-free. Follow up examinations (Fig 7) revealed an entirely normal bladder base in a normal position.

## CONCLUSION

By the use of colpo cysto urethrography urodynamics dissections and anatomically corrective surgery the existence of a specific group of female bladder suspension defects has been proved. The pathology is due to insufficient support of the bladder neck and the anterior part of the bladder base because of laxity of the arcus tendineus fasciae pelvis and/or its firm connection with the bladder neck resulting in a forward downward displacement of the urethro vesical junction. A grading from 1 to 3 is established depending on the involvement of the supporting tissue of the bladder neck and of the anterior vaginal wall. It is shown how tightening of the arcus tendineus fasciae pelvis results in the establishment of a normal bladder base. The bladder base as seen on the radiographs therefore must be considered an indicator of the quality of the support of the bladder and not an indicator of a structure in the bladder wall—a base plate—as advocated by Hutch (7).

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Submitted for publication March 1 1979

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## POSTERIOR BLADDER SUSPENSION DEFECTS IN THE FEMALE

A radiological classification with urodynamic and clinical evaluation

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**Abstract** Of 470 female patients examined by means of colpo-cysto-urethrography 51 patients presented posterior bladder suspension defects. Two distinct forms were seen. 1. Trigonocoele (22 patients)—a downward herniation of the trigone between the postero-inferiorly displaced vagina and the bladder neck, which is retained in a nearly normal position by muscle fibres from the pubococcygeal muscle and the pubovesical ligaments. Symptoms were mostly those associated with prolapse. Stress incontinence was rare while urge incontinence, cystitis and retention of urine were seen. The morphology varied from cases where the hernia disappeared during detrusor contraction (compensated trigonocoele) through typical forms to transitional forms between trigonocoele and posterior bladder descent. 2. Posterior bladder descent (29 patients) comprises postero-inferior displacement of the vagina and bladder base together. Two subgroups are discernible. A. Bladder descent even at rest (16 patients). B. Bladder descent only during micturition (13 patients). Symptoms were varied but stress incontinence was found in 31 per cent in group A and 42 per cent in group B. Morphological forms varied from two cases that were normalized in position during detrusor contraction (compensated descent) to total prolapse during micturition.

Little interest has been focused on the conditions of the female bladder characterized by descent which do not typically cause stress incontinence. Hodgkinson & Doub (4) describe two variants: the cystocoele and the prolapsed bladder, as representing various degrees of relaxation of the support of the bladder and urethra not associated with urinary stress incontinence. Green (3) has the impression that cystocoele and stress incontinence are essentially mutually exclusive. Palm (8) on the other hand finds that cystocoele and bladder descent are bladder diseases accompanied by stress incontinence. One reason for this discrepancy may be that bladder descent consists of two distinct groups. Olesen & Walter (7) by visualization of the vagina with contrast medium have shown that bladder descent is made up of two separate entities: anterior descent and posterior descent.

Anterior descent is not caused by a posterior suspension defect but takes place in front of a normally placed vagina. It is typically accompanied by stress incontinence. This condition will not be further described in this paper. Posterior bladder descent on the other hand is caused by insufficient levator support resulting in a postero-inferior displacement of vagina and bladder together. This condition has a wide spectrum of symptoms. In cystocoele the vagina shows the same displacement as in posterior bladder descent but the bladder neck has not followed the vagina in the displacement. Instead the trigone has herniated down between the bladder neck and the vagina. The present report focuses on these two interrelated conditions constituting posterior bladder suspension defects.

For reasons of clarity the term cystocoele though commonly used in the literature will be avoided because of the double meaning of the word: 1. a bulging anterior vaginal wall demonstrable during a pelvic examination and 2. a herniation of the trigone down behind the bladder neck demonstrable on the radiographs. While the former finding is common and not specific for any particular bladder anomaly the latter is specific from a radiological and urodynamic point of view. Based on the actual findings and to avoid misunderstandings this radiological and urological cystocoele from here on will be termed a trigonocoele.

## METHOD

Colpo-cysto-urethrography i.e. lateral voiding cysto-urethrography with barium contrast added in the vagina was performed with the patient seated. Intravesical pressure was measured transurethrally. The flow was recorded by means of a DISA 14F41 uroflowmeter. Exposures were guided by the flow curve and were marked electronically on the pressure flow tracings.

On a separate occasion the patients were examined clinically gynecologically and urodynamically. This includ-

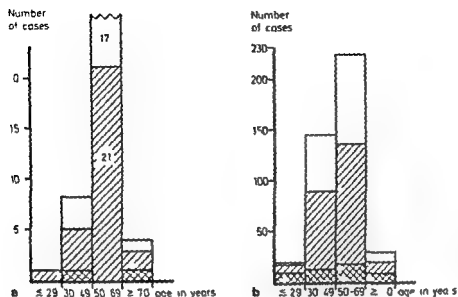


Fig 1 Age and parity distribution of posterior suspension defect patients (a) compared to whole material (b)

water cystometry flow recording and differentiated detrusor pressure measurement.

The two examinations were not compared until independent diagnoses had been reached. The examination technique has already been described in detail by Olesen & Walter (7) and Walter *et al.* (12).

## MATERIAL

One hundred and twenty consecutive examinations were performed and 51 patients displayed posterior bladder suspension defects. Age and parity distribution of these patients are shown in Fig 1. Vaginal repairs had been performed in 16 patients. One patient had had both a vaginal repair and an urethropexy. Primary urethropexies had been performed in two patients and five had had a hysterectomy.

## FINDINGS

**Trigonocoele** Twenty-two patients displayed a trigonocoele during bladder reservoir function, i.e. on the resting and/or cough radiographs. To determine the position of the bladder neck during micturition we measured the urethro-pelvic angle (UP) (Fig. 2) as described by Olesen & Walter (7) and found that it varied considerably. Three patients had very high UP values (above 110°). These patients had all been operated on with urethropexies. Six patients had UP values below 70° indicating bladder descent. Only one

of these patients retained the trigonocoele configuration during micturition while five micturated with the configuration of posterior bladder descent, clearly demonstrating the near relationship between the two types of posterior suspension defects.

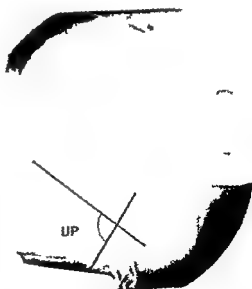


Fig 2 Urethro-pelvic angle (UP). The angle between urethra and a line along the posterior surface of the symphysis through the lowermost part of the obturator foramen closest to the picture.

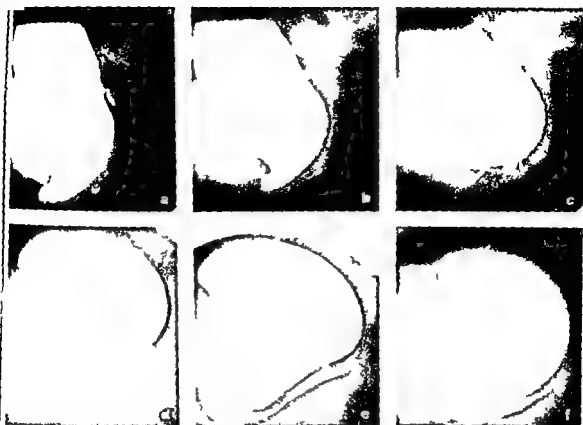


Fig 3 Trigonocoele a at rest b during contraction of the pelvic floor c during micturition of patient who compensates the trigonal herniation during detrusor contraction

d during cough e during contraction of the pelvic floor and f during micturition of a patient who displays a borderline case on posterior descent during micturition

patients elevated the herniated trigone to an entirely normal position and form during detrusor contraction i.e. their trigonocoele was compensated. One patient had a large vaginal cyst distorting the anatomy and one could not micturate during the examination. Thus only eleven patients micturated with the typical trigonocoele configuration and one of these had a low bladder position. They all showed radiological signs of detrusor activity. The internal urethral orifice opened only slightly because of the unstraightened trigone (Fig 3).

Urodynamically they all showed detrusor micturition with elevated intravesical pressure. Flow curves were low and flat. The urethral resistance (intravesical pressure/flow) therefore was higher than normal varying from 0.2 to 4.0 median 0.7. Fridodt Møller (2) gives normal values for the urethral resistance in females between 20 and 30 years  $0.19 \pm 0.16$ . Walter *et al.* (13) found normal values in females between 40 and 70 years to be  $0.15 \pm 0.11$ . The

overall urodynamic picture (Fig 4) corresponds to that of the prostatically obstructed male indicating that the obstruction is near the bladder neck.

Two patients showed signs of detrusor overactivity in the cystometrograms. Clinically the patients all presented with bulging anterior vaginal walls. The symptoms were gynecological complaints 10, straining on micturition 3, retention and cystitis 3, urge incontinence 3 and stress incontinence 3, two of these were due to recurrence after urethropexies i.e. iatrogenic trigonocoeles.

**Posterior bladder descent.** Sixteen patients had low lying bladders even on resting radiographs i.e. the bladder neck was located below the inferior part of the symphysis. The vagina showed a central bulge and a displacement in the postero-inferior direction (Fig 5). Thirteen patients had normally positioned bladders during rest but showed descent during micturition to UP values below 70. One patient did not void during the examination. Two patients lifted a



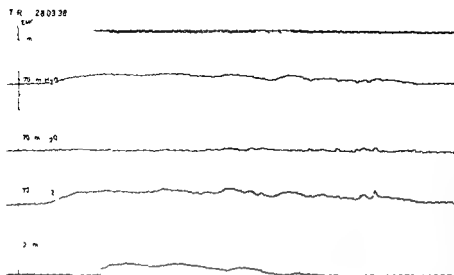


Fig 4 Trigonocoele Urodynamic examination pressure (DP) is calculated electronically by abdominal pressure from total intravesical pressure (IVP)

bladder base to a normal position during micturition i.e. compensated descent. Micturition morphology for the remaining 26 patients were all of the same type but varied in degree of descent from UP values just below 70° through the typical tea pot form to total prolapse of the bladder with the posterior urethra running directly upwards to a sharp bend at the site of the external sphincter. This bend was in all cases the narrowest part of the urethra while the internal orifice was well open.

Urodynamically the pattern was varied and independent of whether the resting bladder was low in

Eleven patients showed the classical descent with undulating flow and pressure tracings (Fig 6) as described by Palm (8). Pressure was almost entirely abdominal and the urethral resistance varied from 0.1 to 4.0 with a median of 0.6. Six patients voided with a normal pressure flow patterns while nine presented a urodynamic pattern as described above for trigonocoele.

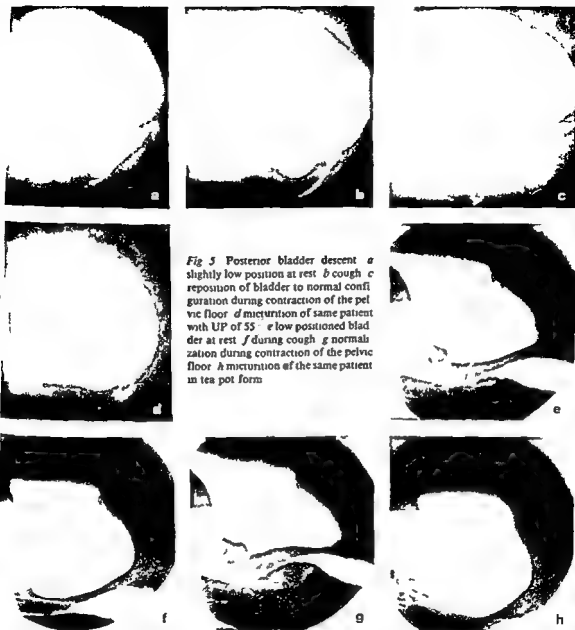
Cystometrograms revealed two cases of uninhibited detrusor contractions. Clinically all had bulging anterior vaginal walls. Symptoms were stress incontinence 13, urge incontinence 8, gynecological complaints 4, straining micturitions 2 and recurrent cystitis 2.

## DISCUSSION

Postero-inferior support of the bladder and vagina is exerted by the levator muscles. This is clearly demonstrated by asking the patient with a posterior descent to contract the pelvic floor. This contraction lifts the

vagina and the bladder base to a normal position. Tanagho & Smith (10) point out that this laxity of the pelvic floor influences the sphincteric function of the urethra and gives rise to stress incontinence. However, only 13 of 29 patients in our study complained of stress incontinence. Tanagho & Smith maintain that the same laxity which causes the low lying bladder may instead cause a trigonocoele uncomplicated stress incontinence as it restores the sphincteric mechanism. Our findings are in agreement with suggestion that a typical trigonocoele is rarely accompanied by stress incontinence. If a patient having trigonocoele is asked to contract the pelvic floor the herniation is not repositioned but merely compressed from behind.

As can be seen from the illustrations the vagina is postero-inferiorly displaced in both posterior bladder descent and trigonocoele but in the latter the bladder neck does not follow the vagina. This creates a space into which the trigone herniates. The bladder neck therefore to some extent must be retained in its original position. Curtis *et al.* (11) state that the medial part of the levator muscle, the pubococcygeus muscle, gives off visceral fibres to the urethra, the vagina and the rectum. Treahy & Pacey (11) describe the same structures and on this finding they base a correctional surgical technique: a pubococcygeus sling behind the urethra. Ingelman Sundberg (5) divides the two pubococcygeus muscles and unites them behind the bladder neck to form a sling. These anterior connections from the pubococcygeus muscle may be intact in trigonocoele and may be partly responsible for retaining the bladder neck. Furthermore the



*Fig 5* Posterior bladder descent *a* slightly low position at rest *b* cough *c* reposition of bladder to normal configuration during contraction of the pelvic floor *d* micturition of same patient with UP of 55 *e* low positioned bladder at rest *f* during cough *g* normalization during contraction of the pelvic floor *h* micturition of the same patient in tea pot form

strong fibro-elastic pubovesical ligaments dissected by Krantz (6) probably partake in keeping the bladder neck from sliding backwards

### CONCLUSIONS

Radiologically trigonocèles may be divided into the following subgroups

1 The compensated form in which the detrusor on

contraction can lift the cele into a normal position resulting in normal micturition

2 The typical form with typical bladder neck outflow obstruction and corresponding urodynamics

3 The transitional form towards posterior bladder descent

4 The iatrogenic form detectable on high UP values  
Trigonocèle symptoms are mostly gynecological  
Stress incontinence is exceptional

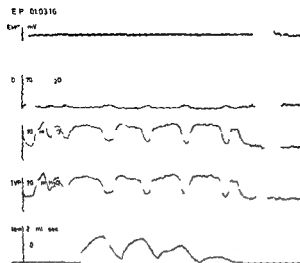


Fig 6 Posterior bladder descent Urodynamic examination

Posterior bladder descent radiologically has the following subgroups

- 1 Primarily low lying bladders that compensate to a normal position and normal micturition during detrusor contraction
- 2 Primarily low lying bladders that remain in the descended position during micturition. These are associated with varied symptoms and varied urodynamic patterns
- 3 Primarily normally positioned bladders that descend during micturition the symptomatology being by stress incontinence Urodynamic patterns varied as with the preceding group

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Submitted for publication March 1 1979

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## SHORT COMMUNICATION

## MEASUREMENTS ON THE HUMAN FETAL UTERUS AND OVARY

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**Abstract** Measurements of the growth rates of fetal endometrium, myometrium and the fetal ovary between 13 to 36 weeks of gestation have been recorded. The relationship of the measurements to the age of the fetuses are presented on the basis of a linear regression analysis. It is concluded that the rates of growth are linear for the fetal uterus as well as the fetal ovary.

The size of the ovary at term and that of the newborn and older child have been studied by various authors (1, 2, 3). It appears that a study of the thickness of the endometrium and myometrium and the size of the ovary during different stages of intrauterine life has not so far been carried out. The present work was designed to study the growth rates of the uterus and ovary. The application of a statistical method can be used to predict the size of the ovary and the thickness of the endometrium and myometrium at any age of the fetus.

## MATERIAL AND METHODS

Forty five human fetuses (from 13 to 36 weeks) from twenty seven therapeutic abortions and eighteen stillbirths were taken for the present study. The stillbirths were taken from cases of abruptio placenta, prolonged labor and cord prolapse leading to fetal death. The age of the fetuses were calculated from the date of the last menstrual period.

The ovaries and uteri were taken out by laparotomy. The length and breadth of the fresh ovaries were measured by vernier calliper of 0.01 least count. The uteri were immediately put in ten per cent formal saline for 48 hours. Paraffin blocks were prepared from the proximal one third of the fundus uteri because the distal two third of the fetal uterus is cervix. Transverse sections of each uterus were made at 7 micra and stained by hematoxylin and eosin. Three measurements from the thickest part of the endometrium and myometrium were taken and the mean values were recorded in millimeters by a Leitz Screw the micrometer eye piece under transmitted light.

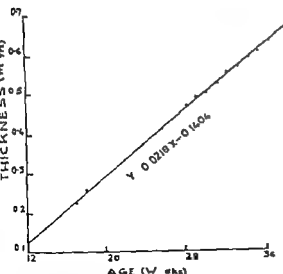


Fig 1 Scatter graph showing the thickness of the myometrium against the age of the fetus along with the fitted regression line.

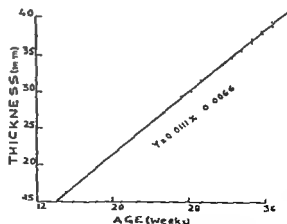


Fig 2 Scatter graph showing the thickness of the endometrium against the age of the fetus along with the fitted regression line.

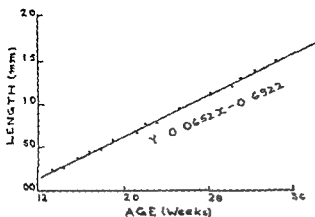


Fig 3 Scatter graph showing the breadth of the ovary against the age of the fetus along with the fitted regression line

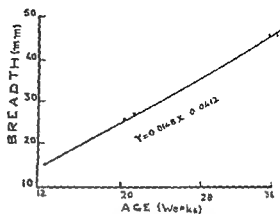


Fig 4 Scatter graph showing the breadth of the ovary against the age of the fetus along with the fitted regression line

### RESULTS

The measurements of the thickness of the myometrium according to age are plotted in a scatter graph (Fig 1) along with the linear regression line. Similar graphs along with the linear regression lines are also presented for the thickness of the endometrium, the length of the ovaries and the width of the ovaries in figures 2, 3 and 4 respectively.

### DISCUSSION

It is clear (Fig 1) that the thickness of the myometrium increases linearly with age and the regression coefficient of 0.0218 was found to be significant ( $p < 0.001$ ,  $F(1, 43) = 47.64$ ) in accordance with the F test based on the analysis of variance. The thickness of the endometrium was also found to increase linearly with age with a regression coefficient of 0.0111 which again was significant. However the rate of growth of the myometrium was found to be much faster than the corresponding rate of growth of the endometrium.

As for the ovary, the length and width were both

observed to increase linearly with age with regression coefficients 0.0652 and 0.0148 respectively which were statistically found to be significant.

### ACKNOWLEDGEMENT

The author is grateful to Dr S K Bhattacharya, Professor of Statistics, Allahabad University for generous help.

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Submitted for publication June 12 1979

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## CASE REPORT

## MONOAMNIOTIC TWINS DIAGNOSED BY ULTRASOUND IN THE FIRST TRIMESTER

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University Clinic Rigshospitalet Copenhagen Denmark*

**Abstract** A case of monoamniotic twins was diagnosed by ultrasonic scanning in the first trimester through the presence of two fetuses in one gestation sac. The fetuses died in utero in weeks 17 and 23. The possible ultrasound appearance of the various types of monozygotic and of dizygotic twins in early pregnancy is discussed and a scheme is suggested which allows some differentiation.

It is well established that fetal life can be reliably diagnosed in ultrasonic scanning as early as a menstrual age of 7 weeks (5). When the gestation sac has been demonstrated, the fetus is located within the sac and the rhythmically moving echoes from the pulsating fetal heart are registered. Since fetal size in early twin pregnancy does not differ from that of singleton pregnancies (2), twin pregnancy should also be diagnosable ultrasonically from the 8th week as two gestation sacs, each containing a fetus. Although this logical assumption seems to hold true in practical work, there are at present no series in the literature to prove it. Conversely, we have encountered an exception to this rule, which we describe here. A case of monoamniotic twins was diagnosed by ultrasound in the first trimester through the presence of two fetuses in one gestation sac. Furthermore, we shall discuss the possible ultrasound appearance in early pregnancy of the different types of monozygotic and of dizygotic twins.

## CASE REPORT

A 25 year old woman, gravida 2, para 2, with diabetes mellitus since the childhood, was routinely referred to the ultrasound laboratory. The first ultrasound examination in the 10th week showed a gestation sac containing a live fetus with a crown rump length of 31 mm, corresponding to 9 to 10 weeks. The next ultrasound examination in week 11 again showed a fetus with a crown rump length (41 mm)

corresponding to the menstrual history, but close to the fetal body we saw a rounded structure 1.5 cm in diameter which changed position in relation to the fetus and had pulsating inner echoes (Fig 1). A repeat examination the following day gave the same result, and a malformed fetus, maybe a monster, was suspected.

Finally the fourth scanning in the 13th week gave the correct diagnosis: two separate fetuses in one cavity. The scanning failed to disclose any membrane between the two fetuses, and since the previous ultrasound examinations had shown only one gestational sac, a diagnosis of monoamniotic twins was made. Fetal growth was normal until week 17, when one fetus died. The other one continued to grow at a normal rate until week 23, when this fetus also died. Abortion was induced by intraamniotic injection of prostaglandin. Two female abortions were delivered: A, 330 grams, 41 cm long and B, 130 grams, 30 cm long. They were lying in one



Fig 1 Transverse scan 11 weeks gestation showing head of fetus (h), body of fetus (b) and round structure (c) with pulsating inner echoes in retrospect transverse section of second fetus.

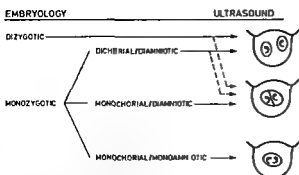


Fig 2 Scheme showing the expected relation between embryology and ultrasound findings in early twin pregnancy. For details see text.

amniotic cavity and had a common placenta. The fetus B had its very thin umbilical cord twisted around the umbilical cord of the fetus A approximately 10 cm from its insertion into the placenta. An autopsy showed no congenital anomalies.

## DISCUSSION

Monozygote twins result from the fertilization of a single ovum, whereas in dizygote twinning two ova are discharged and fertilized in a single ovulatory cycle. Approximately one quarter of all human twins are monozygotic. Depending on how early the separation takes place, one of three different types of monozygotic twins develops (1-3).

1 Separation of the early blastomers results in twins which would start their intrauterine career just as if they were a pair of double ovum twins. They will implant separately and will each develop its own placenta and membranes; i.e. they will be dichorial diamniotic twins (25-30 per cent). At birth the obstetrician would have no means of knowing that they had come from a single egg cell.

2 Duplication of the inner cell mass results in twin embryos which will be enclosed in a single chorion. In addition the placenta is common, but each embryo has its own amnion; i.e. they are monochorial diamniotic twins (70-75 per cent).

3 Duplication of the embryonic rudiment of the germ disc results in twin embryos lying in one amniotic cavity with a common chorion and placenta; i.e. they are monochorial monoamniotic twins (1 per cent). In some of these the separation is incomplete and gives rise to Siamese modifications.

In the second and third trimester monoamniotic twinning may be suggested when scanning fails to disclose a membrane between the two fetuses. Proof cannot be obtained since the membrane may be impossible to visualize with present ultrasound technology.

We were able to diagnose definitely monoamniotic twins in the first trimester. To our knowledge it is the first time this diagnosis has been made by ultrasound. It encouraged us to consider the possible ultrasound findings in early twin pregnancy. In weeks 5 to 7 when the gestational sacs occupy only a minute part of the uterine cavity, three different ultrasound pictures could be expected (Fig 2). A: two clearly separated gestational sacs with a fetus in each would be expected in most dizygotic and dichorial diamniotic twins. B: two gestational sacs with a common wall and with a fetus in each would be expected in monochorial diamniotic twins, and by chance sometimes in dizygotic and dichorial diamniotic twins. C: one gestational sac with two fetuses would be expected only in monoamniotic twins. Clinical evaluation of this theoretical scheme will have to wait until a series of ultrasound examinations in very early twin pregnancy is available.

In monoamniotic twinning the risk of fetal death prenatally or during labor is very high, with a fetal loss of 40 per cent (6), to some extent due to intrauterine death after the 36th week (4). A majority of the fetal deaths is associated with twisting and entanglement of the umbilical cords. When the condition is kept in mind, monoamniotic twinning should be diagnosable ultrasonically from the 8th week, like other types of twinning. The additional information on the exact type allows an adequate choice of the manner and time of delivery (possible preterm) and also suggests the potential of ultrasound in human embryology.

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*Submitted for publication September 6, 1979*

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# FOR OB&GYN LEADERS



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## CASE REPORT

## MATERNAL PULMONARY EDEMA OCCURRING AFTER THERAPY WITH RITODRINE FOR PREMATURE UTERINE CONTRACTIONS

Haim Abramovici Aby Lewin Arie Lissak Abraham Palant

*From the Department of Obstetrics and Gynecology and Department of Cardiology  
Lady Davis Carmel Hospital Haifa Israel*

## COMMENT

**Abstract** Intravenous administration of beta sympathomimetic drugs in combination with betamethasone or dexamethasone is a treatment often used for the suppression of premature uterine contractions and for the stimulations of fetal lung maturity. It has been suggested that the combination of these drugs may be responsible for maternal pulmonary edema and five such cases have been reported in the literature (1, 2).

We report a patient who developed pulmonary edema during ritodrine therapy. Corticosteroids were not given in this case.

## CASE REPORT

A 18 year old gravida II para 0 woman was admitted 33 weeks after her last menstrual period with typical signs of left renal colic.

The patient was treated symptomatically and two hours after admission her pain gradually ceased. Three hours later regular uterine contractions were demonstrated by external monitoring and intravenous administration of ritodrine through an IVAC pump was started (0.1 mg/min).

The patient received the intravenous treatment for 12 hours (total fluids infused 700 cc total ritodrine 150 mg).

The contractions stopped and the patient continued to receive ritodrine orally 10 mg every 3 hours.

24 hours after the beginning of treatment the patient developed dyspnea, tachycardia and on auscultation typical signs of gross pulmonary edema were heard. A chest roentgen ray film revealed pulmonary edema. Digoxin and diuretic treatment were immediately started and over the next few hours all the symptoms disappeared.

The next day the patient was asymptomatic and the roentgen ray chest film was normal.

Phonocardiographic cardiac ultrasonography and the ECG were normal ruling out heart disease.

A few days later the patient was discharged in good condition without further treatment since the contractions had completely stopped and she was asymptomatic. Four weeks later the patient delivered spontaneously a male infant of 3100 grams with an Apgar score of 10. The post partum course was normal.

From the few cases reported in the literature we learn that a beta sympathomimetic drug (terbutaline has been particularly implicated) in combination with steroids can induce pulmonary edema especially when given to a patient with an underlying cardiac disease.

Our case shows that this combination of drugs and/or a previous cardiac disease are not a sine qua non for the development of pulmonary edema. Even in a healthy pregnant woman particularly at 32-33 weeks of gestation when the plasma volume and cardiac output are maximally increased the beta sympathomimetic drug alone can induce pulmonary edema.

We suggest that beta sympathomimetic drugs should not be given to stop premature labor in a pregnant woman with cardiac disease.

Every pregnant woman receiving beta sympathomimetic drugs alone or in combination with steroids should be screened for cardiac disease before treatment and carefully watched during the treatment.

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*Submitted for publication September 6 1979*

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# ANNOUNCEMENT

## INTERNATIONAL AND NATIONAL CONGRESSES 1981

Date	Place	Name	Office
<b>1981</b>			
January 26-31	Mexico City Mexico	Pan American Congress of Andrology	Gerald Bagatzinski Congr Admin 31600 West Chicago Livonia MI 48150 USA
March 14-18	Atlanta USA	37th Annual Meeting of the American Fertility Society	The American Fertility Society 1608 13th Avenue South Suite 101 Birmingham Alabama 35205 USA
March 22-26	Berlin West Germany	IIIrd World Congress of Human Reproduction	Dozent L Mettler Frauenklin der Univ Hegewischstr 4 D 2300 Kiel 1
March 27-28	Modena Italy	Symposium on Recent Advances on Patho-physiology of Amniotic Fluid	Scientific Secretariat Dr G C Di Renzo Istituto di Clinica Ostetrica e Gine- cologica Policlinico Via del Pozzo 71 41100 Modena Italy
April 15-17	Gorizia Italy	International Course on Ultrasound in Obstetrics	Filippo Destro Organising Secretary Dept of Obstetrics and Gynecology City Hospital Via Vittorio Veneto 171 34170 Gorizia Italy
May 17-24	Dubrovnik Yugoslavia	4th European Congress on Ultrasonics in Medicine	Professor Asim Kurjak Ljubinkovac Stube 41000 Zagreb Yugoslavia
June 9-12	Ostend Belgium	Third International Congress on the Menopause	The International Menopause Society 8 av Don Bosco 1150 Brussels Belgium
June 22-26	Jerusalem Israel	5th World Congress of Medical Sexology	Secretariat P O Box 16271 Tel Aviv Israel
Jun-Jul 28-3	Tel Aviv Israel	2nd International Congress of Andrology	Secretariat P O Box 16271 Tel Aviv Israel
July 1-4	Graz Austria	First International Symposium on Minimal Invasive Cancer (Microcarcinoma)	Secretariat First Symposium on Minimal Invasive Cancer P O Box 105 A-1014 Wien Austria
August 20-	Nyborg Fyn Denmark	8th Nordic Congress on Perinatology	Dr Bo Lindberg Dep OB/GYN University Hospital S-75014 Uppsala Sweden
August 24-28	Cambridge England	XIII Acta Endocrinologica Congress	Conference Services Ltd XIII Acta Endocrinologica Congress 3 Bure Street London SW7 3EY England
September 13-16	Nijmegen Holland	International Symposium on Scanning Electronic Microscopy (SEM) of the Reproductive System	Symposium Secretary Gerda Theunissen Department of Obstetrics and Gynecology Sint Radboud Hospital University of Nijmegen 6500 HB Nijmegen the Netherlands
Sept-Oct 28-1	Venice Italy	13th International Meeting Organisation Gestosis	Centro Italiano Congressi Via L. Spallanzani 11 00161 Roma Italy
Sept-Oct	Athens Greece	VIIth European Congress on Sterility (ESCO)	Secretariat Prof Dr K Semm Frauen- klinik der Universität Kiel Hegewisch- strasse 4 2300 Kiel 1 West Germany
October 7-10	Paris France	Third practical course in colposcopy and cervico-vaginal histopathology—in English	Dr Cartier L Enseignement Complé- mentaire en Gynécologie 20 Rue des Cordeliers 75013 France

## CASE REPORT

## ACUTE SPONTANEOUS PUERPERAL INVERSION OF THE UTERUS

Gyvin Skarra and Kaare Leikanger

*From the Department of Obstetrics and Gynecology, Larberg Hospital, Larberg, Sweden*

**Abstract:** Acute inversion of the uterus is a very uncommon but potentially fatal obstetric complication. A case of acute spontaneous puerperal inversion is presented together with survey of data concerning this obstetric emergency.

The complicating condition of uterine inversion, that is, the uterus being turned inside out, is dramatic, serious, and so rare that very few physicians are familiar with it. Having experienced the drama of this unexpected emergency, we feel the need to give a short review of the subject.

Its reported incidence varies greatly from 1/5 000 to 1/250 000 deliveries (1-3). The figures obviously relate to the different standards of general obstetric practice. In most of these cases are due to improper handling of the third stage of labor. Traction of the umbilical cord when delivery of the placenta is delayed, the repeated use of oxytocic drugs when the cervix is very relaxed, fundal pressure in the absence of uterine contraction, uterine tumors and thinning of the myometrium at the site of fundal implantation, and the placenta are etiologic factors. Although doubted by some (3), spontaneous inversion of the uterus has been reported and does occur. It is believed to be due to some abnormality of the uterine musculature and innervation.

Inversion of the uterus is said to be complete when the fundus uteri protrudes through the cervix and into the vagina. Often the entire uterus hangs outside the vulva. The condition usually develops rapidly and is associated with hemorrhage, pain, and shock. The amount of hemorrhage depends on the contractility of the uterus and on the completeness with which the placenta has separated. The degree of shock often appears to be out of all proportion to the amount of bleeding due to stretching of peritoneal nerves and the broad ligament as these structures are often together with tubes and ovaries pulled into the funnel formed by the inverted uterus. Successful treatment depends on the speed of recognition and institu-

tion of therapy. If shock and blood loss are not predominant, the person present at the time occurrence should initiate immediate reposition.

If reposition of the uterus can be accomplished at this stage, shock can be prevented. Early replacement has the advantage that reposition can be accomplished by manipulation before the cervix becomes constricted and grips tightly the encircled portion of the inverted uterus, thus interfering with repositioning and making surgery necessary. If immediate replacement is not possible, treatment of shock and hemorrhage, as well as adequate anesthesia with good relaxation, are essential before manual correction is started. The placenta, if attached, is removed and the uterus replaced vaginally by careful manipulation.

In patients where a cervical ring has developed, the uterus then becoming congested and edematous, it may be quite impossible to accomplish reposition manually, and operation becomes necessary for a successful replacement. Vaginal surgical procedures described by Spinelli and Küstner have been employed successfully, the aim of both operations being to incise the tight cervical ring (1, 2). In the Spinelli operation the bladder is dissected from the anterior uterine wall and pushed out of the way before the cervical ring is divided anteriorly. The inverted uterus is then replaced. The Küstner procedure differs in that the cervical ring is divided posteriorly. Parkley recommends vaginal supracervical hysterectomy as a time-saving procedure (3). Operation by the abdominal route described by Huntington is another alternative (2). The uterus is grasped with Allis forceps about 2 cm below the ring and replaced by steady upward traction under direct vision.

## CASE REPORT

25 years old, para I, gravida II. Her first delivery 3 years earlier was complicated by atonic intra partum bleeding, puerperal bleeding due to retention of the placenta. Ti-

tual delivery at term was spontaneous. 51 E Oxytocin was given 1 m immediately after delivery. 35 minutes after delivery the uterus was found to be totally inverted hanging outside the vulva the placenta still attached to the fundal region. Prior to this the patient had complained of pressure towards the perineum and rectum. Immediate reposition was not tried. Instead the patient was given general anesthesia and about 10 minutes after discovery of the inversion a placenta accreta was removed (histologically verified) and manual repositioning was attempted. However the uterus had become edematous and replacement proved impossible through the firmly constricted cervical ring. Bleeding was profuse the patient shocked blood pressure 40/0 and surgical treatment became mandatory. The cervical ring was cut anteriorly ad modum Spinelli and the inverted uterus replaced. As hemorrhage continued and the uterus remained atonic in spite of oxytocic infusion an abdominal supracervical hysterectomy was performed. Antibiotic coverage was given. The postoperative course was uneventful. Mother and child were discharged from the hospital on the 8th day.

### DISCUSSION

As no fundal pressure or excessive cord traction had been exerted one must assume that this acute inversion of the uterus occurred spontaneously, most probably due to the fundal implantation of a placenta accreta. Her atonic intra partum bleeding and retained placenta three years earlier and associated curettage might also be predisposing factors. Prior to the discovery of the inverted uterus the patient had in fact complained of downward pressure and pain. If a vaginal examination had been done at this point the diagnosis might have been established and the treat-

ment initiated at an earlier stage. Immediate manual replacement was not tried and ten minutes later manual reposition had become impossible as the cervical ring had already formed. Thus it seems that a successful outcome in the management of patients with acute puerperal inversion of the uterus not only depends on correct methods of management but also on early recognition of the problem and prompt institution of therapy.

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*Submitted for publication September 9 1979*

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## CASE REPORT

## SPONTANEOUS RUPTURE OF THE LIVER IN PREGNANCY

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**Abstract** A case of spontaneous rupture of the liver in pregnancy fatal to mother and child is described. The patient, a multiparous 36-year-old diabetic, presented in the 27th week of pregnancy with upper abdominal pains resembling biliary dyskinesia. After 8 hours' observation in the hospital she suddenly developed hypovolaemic shock and fetal heart tones disappeared. On acute caesarean section a dead fetus was delivered. Hemoperitoneum was present, arising from a huge subcapsular hematoma of the right lobe of the liver with rupture of Glisson's capsule. The hematoma was evacuated and tears in the liver parenchyma sutured. Disseminated intravascular coagulation complicated the early postoperative course. A bacterial infection supervened later and the patient died in a septic stage on the 19th postoperative day. The literature is reviewed and it is pointed out that greater awareness of the diagnosis could lead to better timing of surgery and an improved prognosis for mother and child.

Spontaneous rupture of the liver, although rare, is one of the gravest complications of pregnancy with maternal mortality in 59 per cent and fetal mortality in 62 per cent (3). Bis *et al.* (3) recently reviewed the 91 cases known from the literature. They found that the majority had occurred in multiparas in the third trimester and nearly all (85 out of 91 cases) had signs of pre-eclampsia. The true incidence of the condition is unknown, but reported figures vary from 1 case in 40 000 deliveries (7) to 1 in 250 000 (6). In nearly all reported cases the patient is observed in hospital for hours to days with undiagnosed abdominal pain whereupon her condition deteriorates with more severe pain and shock. Emergency laparotomy then yields the correct diagnosis.

The present case, which is believed to be the first reported from Scandinavia, followed this characteristic course.

## CASE REPORT

The patient was 36 years of age. In 1961 a cholecystectomy had been done after 2 years of recurrent gallstone colics. She was then healthy until April 1977 when diabetes mel-

litus was diagnosed; this was well regulated by two insulin injections per day.

**Obstetric history** IV para with normal deliveries in 1961, 1964, 1966 and 1967. These four pregnancies had all been uncomplicated. In particular, there was no record of pre-eclampsia.

**The present pregnancy** Throughout the pregnancy the patient was followed regularly by an internist and the diabetes was well under control. There were no unusual findings until the 26th week of pregnancy when a slight tibial edema and a blood pressure (BP) of 145/95 mm Hg were recorded and controlled with a thiazide diuretic and rest. A week later the patient came to the obstetric outpatient clinic complaining of pain in the epigastric area, irradiating through to the back. No history or signs of physical trauma. Examination showed slight tenderness in the right hypochondrium. No other abnormalities. With a tentative diagnosis of biliary dyskinesia, symptomatic treatment with spasmolytics was prescribed. Late in the evening the same day she returned to the hospital because the upper abdominal pains had increased. On examination she was found to be pale and distressed with pain. Abdomen diffusely tender but without rebound tenderness. Uterus soft, audible fetal heart sounds. No vaginal bleeding. BP dropped for a short while to 100 mm Hg systolic but returned to normal in response to an i.v. drop with Macrodex. The patient was transferred to the obstetric department and observed here for the next 8 hours during which time her condition deteriorated, hemoglobin dropping from 130 to 78 g/l. Acute laparotomy was decided with a preoperative diagnosis of total ablation of placenta. Just prior to operation the fetal heart sounds disappeared so a caesarean section was done. A dead fetus was delivered. The amniotic fluid was clear and the placenta normal. The peritoneal cavity was explored and found to contain 1.5 l of dark blood. The right lobe of the liver was greatly enlarged. A tear laterally in Glisson's capsule was considered to account for the hemoperitoneum. The left lobe and the other abdominal organs were normal. As no fresh bleeding was seen, it was decided to just insert drainage to the affected area and the abdominal wall was closed. However, shortly after the operation fresh bleeding started from the abdominal drains. BP and central venous pressure dropped in spite of blood transfusions. A new laparotomy was decided and within two and a half hours after the first operation the incision was extended to the xiphoid process and revealed that the dome of the right liver lobe was completely covered with a huge subcapsular hematoma, 2 cm thick. Glisson's capsule was torn laterally and medially close to the fal-

ciform ligament. Fresh bleeding oozed from the tears. The hematoma was evacuated and the denuded surface of the liver presented multiple small tears. The liver parenchyma required some hemostatic sutures. Drainage was arranged and the abdominal wall closed. During the two operations BP dropped to 50–60 systolic for periods of 10–15 minutes. A total of 7.5 l of blood were given during the operations.

The postoperative course was complicated. Disseminated intravascular coagulation syndrome was suspected on the second postoperative day because of bleeding from the abdominal drains, thrombocytopenia to  $15 \times 10^9/l$  (normal  $180-350 \times 10^9/l$ ), blood concentration of fibrinogen degradation products above 40 mg/l, oliguria and respiratory insufficiency. Treatment with transfusions of fresh blood, respirator, heparin, chlorpromazine and diuretics produced a slow improvement until the 11th postoperative day when a bacterial infection supervened. Despite antibiotic treatment the patient gradually entered a septic state with unconsciousness, paralytic ileus, oliguria and high fever. She died on the 19th postoperative day.

Post mortem examination revealed a 2810 g liver. Glisson's capsule had been lifted off the parenchyma over an area 8 x 8 cm on the anterior aspect of the right lobe. The underlying parenchyma had multiple small tears. Microscopic examination of the liver revealed zones of cell necrosis. Fibrin thrombi were found in the small periportal vessels. The kidneys showed tubular necrosis and here too intravascular fibrin thrombi were seen. In the lungs there were hyaline membranes in the alveoli and fibrin thrombi in the small vessels. The cause of death was concluded to be disseminated intravascular coagulation combined with hyaline membranes in the lungs and renal tubular necrosis. (Signed: Kerstin Boström)

## DISCUSSION

Rupture of the liver without significant trauma has been known as an obstetric complication since 1844 when Abercrombie (1) reported a case from Cape Town. From time to time since then reports have appeared from all over the world. The exact etiology is unknown, but it is commonly agreed that presence of pre-eclampsia leading to vascular damage of the liver is a main factor. The existence of fibrin thrombi in the liver in many of these cases supports the view that disseminated intravascular coagulation may occur in pre-eclampsia. Normally intravascular fibrin is removed by two mechanisms: fibrinolysis and phagocytosis by the reticuloendothelial system (RES). However, potent activators of fibrinolysis are lacking in the liver and placenta (11). If the RES in the liver is blocked, deposits of fibrin in the hepatic arterioles fanning out into the periportal sinusoids would lead to focal hepatic necrosis. This combined with an

abnormal coagulation profile could be followed by hematoma formation, perhaps precipitated by minor traumas unrecognized by the patient (3). One hypothesis (10) is that pregnancy may sensitize the RES thereby preventing phagocytosis in subsequent pregnancies. This might explain why spontaneous hepatic rupture has been observed mostly in multiparas when primiparas have the higher incidence of pre-eclampsia. That diabetes mellitus may play a role is suggested by 7 patients reported by Hibbard (6) of whom one was a diabetic and two others had clinical evidence suggesting latent diabetes. Apart from a diseased liver, most authors believe that minor traumas which a normal liver would ordinarily withstand might precipitate the formation of a subcapsular hematoma and capsular rupture.

The present case is a good example of the rather characteristic course of events in this entity as described in most reports. The patient usually presents with severe, constant pain in the epigastrium and/or the right hypochondrium. Physical examination reveals upper abdominal tenderness in a few cases also hepatomegaly, but most often at this stage the correct diagnosis is missed and biliary colic (as in this case and many others), pancreatitis (12) and myocardial infarction (5) are usually considered. In a few cases liver scans have contributed to a correct preoperative diagnosis (4) but otherwise the laboratory has provided little assistance. In nearly every case the patient has been under observation in hospital when—hours to days after the onset of symptoms—signs of hemoperitoneum and hypovolemic shock develop as Glisson's capsule ruptures. Even at this stage the exact diagnosis is nearly always missed and the most frequent preoperative diagnoses have been perforating gastric ulcer, occult abruptio placentae or ruptured uterus. Non-operative treatment has been fatal in all cases but one (6). Of 34 patients who underwent laparotomy, 22 survived giving a mortality of 35 per cent (3). In view of the high frequency of coagulopathy (8) it is recommended that blood for coagulation status should be obtained prior to surgery and massive transfusions. For surgical treatment most authors recommend evacuation of the subcapsular hematoma and suturing of tears in the liver with a blunt needle with ligation of bleeding points. In a few cases hepatic lobectomy has been carried out (9). Delivery of the infant by cesarean section at the time of laparotomy is recommended as continuation of the pregnancy has been shown to aggravate the prognosis for mother and child (2, 13).

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*Submitted for publication October 11 1978*

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## CASE REPORT

### CHRONIC GRANULOCYTIC LEUKEMIA IN PREGNANCY

A case report and review of the literature

Oded Shalev Samuel Heyman Giora Hod and Rahel Adato

*From the Departments of Internal Medicine Surgery and Gynecology and the Hematological Service Hadassah University Hospital Mount Scopus Jerusalem Israel*

**Abstract** Chronic granulocytic leukemia (CGL) was diagnosed in the second trimester of pregnancy in a mother of four who decided to terminate the pregnancy immediately. According to the literature this unusual combination is not a definite indication for abortion since CGL does not adversely affect the course of the pregnancy nor the pregnancy alter the course of the disease. Prudent treatment with busulfan or splenic irradiation when indicated apparently does not endanger the development of the fetus.

The occurrence of leukemia during pregnancy has been known since the time of Virchow (12). Following his report in 1845 more than 150 cases have been described of which about half were suffering from CGL (3, 4, 6, 9, 10, 13, 14, 15, 18). The coincidence of the two conditions imposes unique therapeutic problems involving both mother and child. We wish to report a case in which CGL was diagnosed during the second trimester of pregnancy to discuss the therapeutic alternatives and to review the literature.

### CASE REPORT

A 26-year-old mother of four was referred to the Hematological Service during the fifth month of pregnancy because of a white blood count (WBC) of  $5 \times 10^9/L$  found on a routine blood examination. Abnormal physical findings were confined to hepatosplenomegaly. The size of the uterus was consistent with a pregnancy of twenty weeks. Laboratory examinations disclosed a WBC of  $83 \times 10^9/L$ , the differential of which was typical of CGL. Hb 11 g/dl and platelets  $250 \times 10^9/L$ . The morphology of the bone marrow aspirate and the presence of the Philadelphia chromosome in the karyotype confirmed the diagnosis of CGL. After the patient was informed of the nature of her illness she requested that the present pregnancy be terminated and the possibility of future pregnancies be prevented. We therefore

decided to perform in one operation a therapeutic splenectomy, termination of pregnancy (in accordance with the patient's wish) via cesarean section and tubal ligation. No particular surgical problems were encountered during the operation. The fetus was unremarkable. Its bone marrow was normal for a five-month fetus and its karyotype normal unlike that of its mother which was Philadelphia chromosome positive.

During the operation the fetal liver which contains 80 per cent of the hematopoietic activity at this stage of gestation, was extracted and processed for cryopreservation.

The predictable post-splenectomy thrombocytosis of up to  $750 \times 10^9/L$  which developed after the operation was treated with 300 mg sulfapyrazone per day until the surgical wound healed, whereupon cytotoxic therapy with thioguanine and hydroxyurea was substituted. This effectively controlled the CGL. The patient has been maintained on thioguanine and hydroxyurea and her condition has remained hematologically stable over the past six months.

### DISCUSSION

Following the original description of leukemia during pregnancy in 1845, Sheehy (15) collected 89 cases of CGL occurring during pregnancy that had been reported up till 1958. In 66 of them the CGL had been diagnosed prior to the pregnancy and during pregnancy in the other 23. The next large series on the subject was reported by Moloney (10) who collected 69 cases of concurrent leukemia and pregnancy between 1958 and 1963. 19 of the 22 women with CGL bore normal infants. Ask-Upmark (1) reported another 3 women with CGL who had normal pregnancies and whose infants were normal. Still another patient was first found to have CGL during the seventh month of her pregnancy. She received no treatment and the pregnancy terminated in the birth of a normal child (7). Fourteen months after the diagnosis she became pregnant again and this time opted for a therapeutic abortion.

The disease seems to have no deleterious effect on the offspring. The child born to a mother suffering from CGL was followed for 20 years and found completely normal. The CGL was diagnosed when the mother was in the first trimester of pregnancy and treated with splenic irradiation; the pregnancy was terminated by cesarean section because of eclampsia (11). For a period of more than 30 years Bjure (2) followed the children and grandchildren of another patient with CGL; again no trace of the disease was found. Likewise pregnancy does not seem to change the natural course of CGL. The median survival of 44 women with CGL who became pregnant during the course of their disease was 38 months (12). This mean is not significantly different from that of the rest of the adult CGL population (17).

The current cytotoxic treatment available for CGL is a potential menace to the developing fetus (16). It is therefore recommended that therapy be withheld if possible at least until the end of the first trimester. If however the patient develops signs of accelerated activity of the disease such as a very high WBC and massive splenomegaly, specific anti-leukemic therapy should be instituted. Busulphan, the classical drug for CGL (8), appears to be the safest drug under these circumstances. The newborns of 12 women with CGL who were treated during their pregnancy with busulphan were all normal, even though 10 of the mothers received the drug during the first trimester (9, 13). Moloney's review (10) includes another 12 women who were treated with busulphan during the course of their pregnancy and in 6 of them this drug was the only therapy administered. Only one of the women gave birth to a malformed child (5). Although busulphan was implicated in this case, the patient also received 6-mercaptopurine and splenic irradiation in the first trimester. Another cytotoxic drug, dibromomannitol, was successfully used between the 10–17 weeks of a pregnant woman with CGL. The child was normal at birth and remained so during the four year of follow up (3).

Although the experience with splenic irradiation is limited, it appears to be a safe therapeutic alternative, especially when the CGL is diagnosed early in the pregnancy and the uterus is still small enough to be shielded (14).

Our patient, a mother of four children, preferred to terminate the pregnancy. The relatively advanced stage of the pregnancy, her wish to prevent further pregnancies and our policy to perform therapeutic splenectomy in the early stages of CGL (19) dictated

that all the surgical procedures be done in one operation: abortion via hysterotomy, tubal ligation, splenectomy. The post-splenectomy patient was treated with the anti-platelet agent sulfinpyrazone and as soon as the surgical wound had healed, definitive therapy with the cytotoxic agents hydroxyurea and thioguanine was instituted.

In summary, CGL does not appear to affect adversely the course of the pregnancy nor the fetus. Moreover, there is no evidence that the pregnancy alters the natural course of the disease nor the mean survival in mothers with CGL. In spite of the relative safety of the treatment of CGL, it is recommended that it be deferred until at least the second trimester unless the mother's health is endangered by an otherwise uncontrolled activity of the disease. The experience, primarily with busulphan and splenic irradiation, although limited, is the best available to date.

#### ACKNOWLEDGEMENT

The authors thank Professor E. Rachmilevich for referring the patient and reviewing the manuscript.

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*Submitted for publication in November 20 1979*

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## ANNOUNCEMENT

**The Pan American Congress of Andrology** will take place in Mexico City Americana Fiesta Palace Hotel January 26–31 1981

*Information requests for registration and travel information should be directed to*

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**The 11th World Congress of Human Reproduction** is to be held in West Berlin March 22–26 1981

### *Main topics*

- Central nervous system and regulation of reproduction
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- Beginning of life *in vivo* and *in vitro*
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### *Information*

Congress Secretariat  
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**The Third International Congress on the Menopause** will be held in Belgium at the coastal resort of Ostend in June 9–12 1981 just before the holiday season begins

This Congress will differ from the previous two (in La Grande Motte 1976 and in Jerusalem 1978) in that where as the aim in the past was to arrive at a consensus of opinion this time we shall bravely attempt to confront the controversies. It will however take the same form as the earlier ones — a number of small workshops rather than one large meeting attended by everyone

### *For further information*

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B-1150 Brussels  
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Tel (02) 771 98 98 and (02) 771 98 45

**An International Symposium on Scanning Electronic Microscopy (SEM) of the Reproductive System** will be held in Nijmegen The Netherlands September 13–16 1981

The Symposium will consist of sessions (1) fundamentals of SEM preparations (2) reproduction morphology (3) reproductive pathology (4) advanced techniques and clinical application

Each will comprise invited lectures and free communications. Keynote lectures will include Hans Ludwig Godfried Roomans Eva Patek James Koehler Kenneth Gould Laurens Zaneveld *et al*

Selected and extended abstracts will be published. The deadline for submission of abstracts is April 1 1981

*Further details may be obtained from*  
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6500 HB Nijmegen  
the Netherlands  
Phone 80-514725

## CASE REPORT

### LIVER CELL CARCINOMA IN YOUNG WOMEN POSSIBLY INDUCED BY ORAL CONTRACEPTIVES

Flemming Amtrup Jørgen Slottved and Hans Svapholm

*From the Institute of Pathology and the Department of Neurology Vejle Sygehus Denmark*

**Abstract** Two cases of liver cell carcinoma in young women using oral contraceptives have been found within a few months in the county of Vejle Denmark. Including these two patients 22 cases of liver cell carcinoma in young women have been published since 1973 19 of whom have used oral contraception. The possible effects of oral contraceptives in inducing liver tumors is discussed and it is pointed out that no statistical proof of such an effect exists at present.

Primary hepatic cancer is a relatively frequent disease in Asia and Africa (2-21) but is uncommon in Western countries. It is most often seen in the middle aged and in men (28).

However in Denmark the incidence of primary hepatic cancer exclusive of sarcoma has varied from 4 to 5.0 per 100 000 age adjusted European standard population per year among men from 1943 to 1972 and among women the corresponding figures are 2.9 to 6.3 the latter incidence being for the period 1943-1947 (8). Liver cell carcinoma is frequently combined with cirrhosis of the liver the recorded incidence varying from 17 per cent to 70 per cent (12-21).

During recent years a possible connection between oral contraception and liver cell carcinoma has been discussed. A connection between liver cell adenoma or focal nodular hyperplasia and oral contraception was postulated by Kay and Shatzki (19) (19) Hovath Kovacs and Ross (17) and by Baum Bookstein Holtz and Klein (1973) (3). In September 1976 117 such cases were published and 120 additional cases mentioned (but not proved) in the literature or 237 cases in all (20). The diagnosis were liver cell adenoma (3-9-11) one hepatoblastoma (23) focal nodular hyperplasia (24-30) and possibly one hamartoma (26).

Since 1975 several cases of liver cell carcinoma in young women taking oral contraceptives have been published (5-13-14-15-16-20-22-32) a total of 20 cases up till July 1978 (7). Among these women 17 had taken oral contraceptives for from 6 to 120 months and all the cases have been without cirrhosis (7-20).

## CASE REPORT

**Case 1** A 22 year old previously healthy woman was admitted to hospital in July 1978 six months post partum because of gastroenteritis which had lasted 111 days. She developed an encephalitis-like disease and died.

**Sterile autopsy** In the right lobe of the liver a tumor with a greatest diameter of 15 cm was found. No virus was cultivated. Presumptive cause of death was hepatic coma.

**Microscopy of the tumor** Liver cell carcinoma. No cirrhosis.

**Gynecology** During her sixteenth and seventeenth years she had used oral contraceptives containing both estrogen and gestagen name unknown. Normal delivery March 1977. She had used Mini Pe (0.3 mg norethisterone daily) before the delivery and six months after.

**Case 2** A 20 year old previously healthy woman was admitted to hospital in October 1978 six months post partum because of a wish for abortus provocatus and because of pain in the epigastrium in which region a palpable tumor was found. After ultrasonic scanning a solid tumor in the liver was suspected. At laparotomy a tumor with a greatest diameter of 9 cm was found in the left lobe of the liver and hemihepatectomia sinistra was carried out. No postoperative complications.

**Microscopy of the tumor** Liver cell carcinoma. No cirrhosis.

**Gynecology** Menarche 1975. From February 1975 to February 1977 she had used oral contraception (Ovanone 7 tablets with 40 microgram ethinylestradiolium 15 with 2.5 mg lynestrenolium and 50 microgram triadolum). Normal delivery April 1978.

## DISCUSSION

Since the beginning of this decennium the number of liver cell adenomas and focal nodular hyperplasias published in the literature from several western countries has increased dramatically.

It has been suggested that liver cell adenoma and focal nodular hyperplasia represent two phases of the same disease which may develop into liver cell carcinoma (10-20).

There has been an increasing interest in sex steroids and contraceptives as a possible cause of liver tumors (3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 18, 20, 22, 23, 24). It is well known that sex steroids and oral contraceptives change the ultrastructural picture and function of the liver (1, 29).

It has been stressed that women who use oral contraceptives may show dilatation of the periportal sinusoids which may look like peliosis hepatis (33). The same change has been described in many of the cases published in which young women ingesting oral contraceptives have developed hepatic tumors (9, 10, 13, 24) and in our case 1 dilatation of sinusoids and small veins was found.

In both our cases the intake of oral contraceptives was of short duration: patient 2 using Ovanone for only 2 years while patient 1 had used an unknown contraceptive of the usual combination type for only 1-2 years and after that used a gestagen pill for six months before and six months after her pregnancy. It is remarkable that both the tumors were diagnosed post partum and in case 2 furthermore in early pregnancy. Previously hepatic tumors have been found in connection with pregnancy or the puerperium among women not using oral contraceptives (4, 6). One more case of liver cell carcinoma found during pregnancy has been published but previous to her pregnancy this patient had received longterm treatment with estrogen like tablets (31). In our case 2 we found deposits of alpha 1 antitrypsin in the tumor cells which Palmer *et al* also found in 8 out of 9 of their cases of malignant hepatoma.

According to the Danish Cancer Registry the number of malignant hepatic tumors exclusive of sarcomas in men and women has not increased from 1943 to 1972: the last half of the period coinciding with the general use of oral contraception among women (8).

According to Christopherson and Mays the National Cancer Institute has calculated that about 35 million women in USA have used or do use oral contraceptives (6). Since 1973 22 cases of liver cell carcinoma

of young women including our two cases are on record. Of these cases 19 have used oral contraceptives. The risk of contracting liver cell carcinoma by using oral contraceptives. The risk of contracting liver cell carcinoma by using oral contraception must therefore be extremely small if there is any connection at all. Previous to the general use of oral contraceptives liver cell carcinomas occasionally were found in young people also in women. How important oral contraceptives are among presumably many other pathogenic factors is still unknown. There is still no statistical evidence that oral contraceptives are causative.

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Submitted for publication June 25 1979

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## LETTER TO THE EDITOR

### PERINATAL ASPHYXIA IN SPITE OF A NORMAL CARDIOTOCOGRAM AND A NORMAL ACID BASE STATE AT THE TIME OF DELIVERY

Dear Sir

In the case described by Dr T Weber (Acta Obstet Gynecol Scand 59 371-373 1980) the cardiotocogram is said to be essentially normal.

Except for the initial 14 minutes (Fig. 1) and the last 25 minutes (Fig. 3) the FHR baseline shows in my opinion a rather pronounced silent pattern which in this case should arouse suspicion of acidosis corroborated by the depressed HPL values. The FHR anomalies related to contractions described as early decelerations show on 8 occasions initial and/or terminal accelerations raising a suspicion of umbilical cord pathology.

The neonatal depression of the newborn might have been caused by placental insufficiency (low PL, silent pattern) and pathological umbilical cord circulation (variable decelerations).

Against this background the statement 'No infectious anomalies or other explanations for the per-

inatal depression were found' is too cursory. Was the case really scrutinized for any pathology regarding the umbilical circulation? Was the placenta investigated histologically?

October 27 1980

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Submitted for publication October 27 1980

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## LETTER TO THE EDITOR

*Dear Sir*

In a recent issue (59 371) of *Acta Obstetrica et Gynecologica Scandinavica* author T. Weber presents a case of perinatal asphyxia in association with normal cardiotocogram.

Since the interpretation of the cardiotocogram during the second stage of labor is essentially different from our clinical practice, I wish to make some remarks on decelerations. Dr. Weber regards the decelerations (varying in size and shape<sup>1</sup>) as early decelerations—which should be uniform (as in Fig. 1). According to our concept, the decelerations in the second stage are typical variable decelerations indicating an umbilical compression, which also explains the low Apgar score at one minute. The rapid recovery of the newborn accords with this concept. I hope that readers of the journal will benefit from this

comment as an example of the difficulties encountered when making a visual interpretation of cardiotocograms.

Yours sincerely

V. Kariniemi

*Submitted for publication*

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## LETTER TO THE EDITOR

Reply to the letters of Drs Karimemi and Jenssen regarding my paper in *Acta Obstetrica et Gynecologica Scandinavica* (59 371 1980) I have the following comments concerning the cardiotocogram (CTG) of the second stage of labor (Fig 3)

Although the decelerations during the short (10 min) second stage of labor might be interpreted as variable decelerations they are in principle early decelerations (caused by head compression) as each bearing down-effort is seen together with a drop in the fetal heart rate (FHR). This drop is the more pronounced the higher the intrauterine pressure is during the bearing-down-effort.

Umbilical cord compression causes respiratory acidosis with acute accumulation of  $\text{CO}_2$  and an increase in the A-V difference in pH and  $\text{pO}_2$  of the blood of the umbilical cord vessels (3). The acid base parameters in the discussed case thus did not indicate cord compression as  $\text{pCO}_2$  of the umbilical artery and vein was normal (7.5 and 6.1 respectively) and as the A-V difference in pH and  $\text{pO}_2$  of the umbilical cord vessels were normal (0.07 units and 1.4 kPa respectively).

Even if the second stage decelerations are called variable they are mild variable decelerations (decrease in FHR less than 60 beats per minute) (1). Consequently the low Apgar score in the present case cannot be explained by the decelerations as mild variable decelerations during the second stage of labor are not associated with low Apgar scores (1).

No abnormal position of the umbilical cord was found at delivery: the length of the umbilical cord was normal and the placenta was normal although not investigated histologically.

Concerning the CTG during the first stage of labor (Figs 1 and 2) I have the following comments:

- 1) The variability was normal (never less than six in frequency (oscillations per min) and amplitude (beats per min)) (2).
- 2) Although it is a matter of debate whether the decelerations during the first stage of labor are variable or early they are mild/moderate and

never severe (2). According to Krebs *et al* (2) the CTG should thus be described as uncomplicated with mild/moderate variable decelerations: a pattern not related to low Apgar scores one (and five) minutes after delivery.

- 3) The CTG did not change after tissue pH had reached normal values.

In conclusion this discussion demonstrates the difficulties in interpreting a cardiotocogram. In my opinion this problem can only be solved when tissue pH monitoring can be performed during all CTG monitored deliveries.

November 27 1980

Tom Weber

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Submitted for publication November 27 1980

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